Austrian 3Rdays

2026

Building Bridges
Bursting Bubbles

Vienna

April 28 - 30, 2026

ABSTRACTBAND

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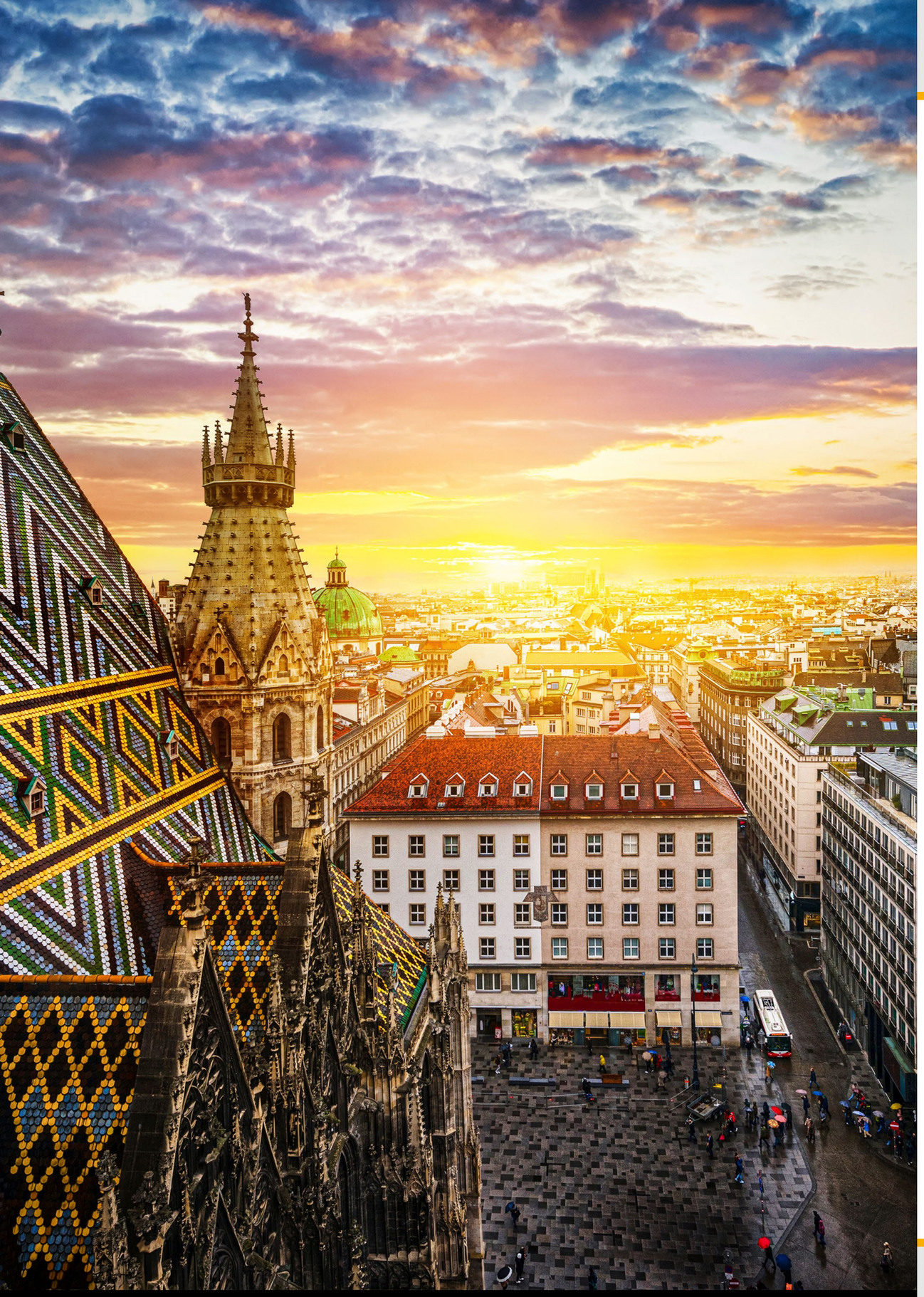

DATE

April 28 - 30, 2026

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OPENING

Abstract Number: 167

Presentation

EVIDENCE-BASED TRANSITION TO ANIMAL-FREE INNOVATIONS

Merel Ritskes-Hoitinga[1]

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Since qualifying as a veterinarian in 1986, I have worked to improve laboratory animal welfare and scientific quality for better human translation. I began implementing the 3Rs - especially Reduction and Refinement - but found that 3Rs alone were ineffective. I pivoted to systematic reviews to expose duplication and drive 3R uptake. These, and a translation scoping review, revealed the inability to predict whether animal findings translate to humans. This evidence shifted me to human relevant New Approach Methods (NAMs) - organoids, organ on chip, in silico modeling, and AI - grounded in human tissues and data. Multiple examples now show better human translation with NAMs than with animal studies. Historical analyses indicate many animal testing mandates were crisis driven after drug disasters, not evidence based. COVID 19 further showed timelines can shrink via NAMs, reduced animal testing, data sharing, reuse of historical data, and earlier clinical trials.

In the Netherlands, I serve on the Transition to Animal Free Innovations program. Using transition and social science insights, we study how to phase-out animal studies and scale-up NAMs through an interdisciplinary coalition across academia, industry, policy, and regulators. Within the SAFE consortium (Safety Assessment through animal Free Evolution), we map barriers and enablers to regulatory NAM uptake and chart pathways to accelerate human relevant science and NAM adoption. The presentation will include case studies.

RETHINKING ANIMAL RESEARCH: QUALITY, WELFARE & THE 3RS

Abstract Number: 10

Presentation

PRACTICAL ADVICE ON IMPROVING QUALITY, SAFETY AND TRANSLATABILITY IN ANIMAL FACILITIES

Adrian Smith[1]

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Plans for animal research have often been through a rather academic and theoretical process before scientists ask an animal facility to start a project. Funding organisation panel members and authors of guidance on experimental design may well be experts in their field, but they are not necessarily experts on the management of an animal facility, with the potential this has to affect quality.

Scientists and facility staff may thus end up debating best practice. Close cooperation should be encouraged from day 1 of planning, if there is to be hope of achieving Replacement. A Culture of Care will ensure that doubts and "stupid questions" can be raised without fear of ridicule or reprisal. This may also prevent serious accidents: the majority of which are the result of a cascade, or simultaneous occurrence, of a number of events which, individually, are relatively harmless. Staff should be encouraged to embrace small incidents as a means of preventing larger ones, rather than ignoring them or covering them up. The research animal world has much to learn from industries with systems for minimising and investigating accidents. On the positive side, even small improvements can lead to large gains in quality, welfare and safety.

Norecopa address these issues, building on experience from AAALAC-accredited facilities and LAS courses. This includes the PREPARE guidelines, and 10,000 webpages of links to 3R resources.

Conflict of interest: Norecopa is a member of AAALAC (one-time payment)

Abstract Number: 2

Presentation

A PARADIGM SHIFT IN INTERPRETING THE 3R PRINCIPLE

Herwig Grimm[1], *Marc Dusseldorp*[2]

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The 3Rs are generally understood as principles – but not taken seriously as such, as a normative theoretical reflection based on Robert Alexy shows. Principles are imperatives for optimisation and, as such, require that their content be realised to the greatest possible extent relative to the normative and actual possibilities. This reflected normative character of the 3Rs results in a paradigm shift in their interpretation with far-reaching implications for practical application. By replacing the common 'improvement paradigm' with the 'deviation paradigm', the 3Rs can only develop their full effect for laboratory animal protection – in line with Russel & Burch and EU Directive 2010/63. The presentation, which is based on a previously published paper („The (re)turn of the 3Rs" (Grimm & Dusseldorp 2025), DOI: 10.1177/00236772251326352), will cover the theoretical background as well as an ethics tool to implement the 3Rs according to the deviation paradigm im practice.

Abstract Number: 145

Presentation

DO YOU C.A.R.E. FOR LABORATORY ANIMALS? A WELFARE LABEL FOR THE CRITICAL ASSESSMENT OF REFINEMENT IN EXPERIMENTS

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Despite decades of ethical debate and regulatory progress, animals used for research still endure considerable cost and harm. Innovations for the benefit of animals are incremental, and their implementation is very slow. To address this issue, we propose introducing a voluntary welfare label called CARE (Critical Assessment of Refinement in Experiments). This initiative is based on the principle that laboratory animals should be offered a life worth living, characterised by positive experiences, social engagement, autonomy, and opportunities for play, stimulation, and development. The CARE label will formally recognise and reward papers of researchers and research groups that proactively improve the welfare of their laboratory animals beyond minimal requirements. The label would focus on three major domains of refinement: husbandry, procedures, and the human–animal relationship. A three-tiered award system with bronze, silver, and gold medals will recognise the extent and ambition of the implemented refinements. This approach represents a shift in focus from enforcing minimum standards to incentivising improvements of animal welfare in scientific research. In this paper, we outline the conceptual basis, implementation, and potential of the CARE label regarding animal welfare, scientific quality and the societal acceptance of animal research.

NAMS & 3RS IN INFECTIOLOGY (THE IGNAZ SEMMELWEIS INSTITUTE SESSION)

Abstract Number: 169

Presentation

BEYOND THE ANIMAL MODEL: THE PIG AS A 3R PLATFORM FOR VACCINE RESEARCH

Tobias Käser[1], Juan Bernardo Odasso[1], Leonie Bettin[1], Christine Unterweger[1], Andrea Buzanich-Ladinig[1], Doris Wilfingseder[1]

[1] Vetmeduni Vienna, Vienna, Austria

Due to their close physiological and immunological similarity to humans and their importance within a One Health framework, pigs are a highly relevant model for translational immunology and vaccine development. At the same time, they offer unique opportunities to integrate the 3R principles.

Our approach builds on the role of pigs in food production. In collaboration with a slaughterhouse, we obtain primary porcine tissues (e.g., blood, genital tracts, and skin) as by-products of food production and use them to establish advanced in vitro systems, such as transwell cultures. We also generate extracellular matrix scaffolds using pig tissues – e.g. collagen type I from pig skin. This creates physiologically relevant allogeneic matrix platforms without the need for dedicated animal sacrifice.

When in vivo studies are required, we maximize information gained per animal. The size of pigs enables extensive longitudinal sampling, yielding large quantities of immune cells and endpoint tissues that are cryopreserved and reused. This supports complex downstream applications, including co-cultures of antigen-specific T cells with autologous infected epithelial cells, allowing detailed studies of host–pathogen interactions while minimizing animal use.

Using this integrated strategy, we investigate immune responses and develop vaccines against pathogens such as Chlamydia trachomatis, combining translational relevance with a scalable 3R framework for biomedical research.

Abstract Number: 149

Presentation

THE DAWN OF A NEW ERA:ADVANCING SCIENTIFIC VALIDITY PRE-CLINICAL RESEARCH WITH STEM CELL-BASED HUMAN MODELS

Magdalena Erlacher[1], Doris Wilflingseder[1]

[1] Ignaz Semmelweis Institute, Interuniversity Institute for Infection Research, Vetmeduni, Vienna, Austria

Langerhans cell Histiocytosis (LCH) is a genetic malignancy mainly caused by a mutation in the Braf gene (BRAFV600E). LCH may affect people at any age but is mainly occurring in small children, with a peak incidence at the age of 1 to 3 years. Although being highly heterogenous, LCH is characterized by an infiltration and accumulation of immature Langerhans cell type dendritic cells (DCs), majorly affecting skin, lung and bone. Due to its rare occurrence rate, understanding of the ontogeny and pathogenesis of the disease remains a challenge. Up to now the use of animal models for testing potential drugs or therapies, or for studying the mechanism of the disease seem inevitable. In consideration of the 3Rs principle we established a pre-clinical model by use of umbilical-cord blood derived CD34+ stem cells. The introduction of a BRAFV600E mutation into human HSPCs enabled us to investigate signaling pathways crucially involved in the development of LCH, to identify phenotypical and functional differences compared to normal epidermal LC differentiation and to investigate transcription factors known to be activated in LCH. Confirmation of in vitro gained data with primary LCH patient biopsies demonstrated the faithful reproduction of BRAFV600E-driven effects in engineered stem cells. In accordance with the 3Rs principle, this approach does not only overcome interspecies variations but also empowers new possibilities and opens high flexibility in immune cell research.

Abstract Number: 49

Presentation

DIFFERENT 3D APPROACHES TO CULTURING BOVINE SKIN: EXPLANT CULTURE VS. ORGANOTYPIC SKIN MODEL

Christina Baumbach[1], Nadia Anantama[2], Vuk Savkovic[3], Christoph Mülling[2], Jan Schinköthe[2], Jule Michler[1]

[1] University of Veterinary Medicine Vienna, Dpt. of Biological Sciences and Pathobiology, Vienna, Austria
[2] Faculty of Veterinary Medicine, Leipzig University, Leipzig, Germany
[3] Department of Oral and Maxillofacial Surgery, Leipzig University Medical Center, Leipzig, Germany

Bovine digital dermatitis (DD) is an infectious skin disease in cattle leading to painful ulcero-proliferative lesions. Although DD has a major impact on animal welfare and causes substantial economic losses, the knowledge on its pathogenesis is still limited. Not least due to insufficient animal experiments, in vitro skin models represent a valuable tool to accelerate the study of DD.

Two in vitro skin models using bovine skin from the interdigital cleft of the distal limb were established: explant cultures and organotypic models composed of previously isolated primary keratinocytes and fibroblasts.

For the explant model, full-thickness skin samples were cultured at air-liquid interface for up to 7 days. The organotypic model consisted of two parts: layered keratinocytes resembling the epidermis reside on a dermal equivalent consisting of bovine collagen and mitotically inactive fibroblasts. The latter could be maintained for up to 21 days. At regular intervals, samples of both models were taken for histological and immunohistochemical analysis.

Explant cultures showed ballooning degeneration of keratinocytes and segmental necrosis starting at day 5. Both incorporated cell types of the organotypic model showed typical lineage and differentiation markers, epidermal stratification and initial cornification could be observed.

Both in vitro models proved dependable, each with its own benefits, and constitute a viable option for replacing experiments on live animals.

Abstract Number: 96

Presentation

FINDING THE GOOD IN THE BAT - CHARACTERIZATION OF VIRUS-HOST INTERACTIONS AT BAT RESPIRATORY BARRIER SITES

Babette Fletemeyer[1], Magdalena Erlacher[1], Doris Wilflingseder[1]

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Not only since the SARS-CoV-2 pandemic have bats become an interesting order to study. Bats are known reservoirs for several zoonotic viruses such as Ebola, Marburg, Hendra, Nipah and coronaviruses. Interestingly, bats can tolerate many viral infections without showing disease symptoms.

Previous research has highlighted differences in the complement system, interferon expression and immunoglobulin genes as possible explanations for bats' effective coping mechanisms compared to humans. However, functional and comparative analyses at the airway mucosal interface, the primary site of viral entry, remain limited.

To address this gap, I will establish standardized in vitro airway models derived from different bat species and humans. Airway organoids cultured under submerged conditions will enable high-content screening, while air–liquid interface cultures will model the respiratory epithelium in a more physiologically relevant setting. Using both systems, I will compare innate immune responses during viral infection, focusing on the activation and regulation of the complement system as an early line of defense.

Establishing robust in vitro models for bat respiratory infection will contribute to the replacement of animal experiments in infection research. The insights gained will advance our understanding of bat immunity and may help develop new strategies for treating severe viral infections in humans.

Abstract Number: 108

Presentation

HUMOR: A HUMAN MONOCYTE-DERIVED DENDRITIC CELL–ORGANOID MODEL FOR SARS-COV-2 INFECTION

Paul Schweighofer[1], Doris Wilflingseder[2], Lukas Alfons Huber[3], Wilfried Posch[1]

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[3] Institute of Cell Biology, Biocenter, Medical University of Innsbruck, Innsbruck, Austria

Animal-derived components result in batch-to-batch variability in in vitro immune models that limit reproducibility and translational relevance. In line with the 3R principles, we established an animal-free human in vitro model to investigate and characterize dendritic cells (DC) in the context of SARS-CoV-2. Monocytes were differentiated into monocyte-derived DCs (moDCs) using human platelet lysate (hPL) to substitute fetal calf serum (FCS) as a serum component. hPL-derived moDCs showed robust differentiation and reduced baseline activation, enabling improved signal-to-noise ratios during viral stimulation. MoDCs were integrated into a human lung organoid co-culture system generated under animal-free conditions to mimic the lung environment, presenting structural integrity in both cellular components' phenotypes. Using confocal imaging-based quantification, we assessed DC activation marker expression following exposure to different SARS-CoV-2 variants. This animal-free platform reduces experimental variability and provides a physiologically relevant system for studying DC–virus interactions at the lung barrier. Our approach demonstrates the feasibility of advanced immune-infection modeling without animal components and supports the implementation of 3R-compliant strategies in infectious disease research.

Abstract Number: 168

Presentation

FLOW, FUNGI, AND FIREWALLS TO RESPIRATORY CHALLENGES

Doris Wilflingseder[1]

[1] Vetmeduni Vienna, Ignaz Semmelweis Institute, Infectiology and Virology, Vienna, Austria

Our work outlines progression from engineered microenvironments to disease-relevant co-cultures to dissect airway epithelial–immune crosstalk. Microfluidic platforms (Chandorkar et al., 2017) established controlled flow, gradient formation, and barrier architectures that support reproducible assessment of epithelial integrity, signaling, and trafficking under physiologically relevant shear. Building on this, studies of epithelial interactions with fungi (Luvanda et al., 2021a,b) characterized sentinel epithelial functions and mapped bidirectional signals that recruit and instruct innate effectors during early antifungal responses.

These concepts were extended to viral infection in co-cultures examining epithelial–macrophage/neutrophil interactions with SARS CoV 2 in healthy and cystic fibrosis (CF) airway epithelia (unpublished). The data indicate that epithelial genotype and baseline barrier/inflammatory status modulate antiviral set points, shaping infection programs, and inflammatory output. Differential inputs from macrophages and neutrophils further refined outcomes, highlighting cell type-specific contributions to protection and pathology.

Together, these studies provide a modular, flow-aware framework for analyzing mucosal defense across pathogens, linking biophysical context to immune function. The approach supports standardized readouts, comparative analyses across disease states, and the identification of mechanism-based targets and biomarkers for intervention.

Abstract Number: 22

Poster

GENERATION OF A PORCINE 3D CELL CULTURE MODEL TO MIMIC COLITIS IN VITRO

Katrin Spirk[1], Maik Dahlhoff[1]

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Colitis describes an inflammation of the large intestine, causing mild to severe symptoms that can heavily impact the quality of life. To study the cause, progression or treatment for colitis, animal models display an in vivo model, nonetheless with several drawbacks like the poor translation from animals to humans. In line with the 3R approach, there is a desired need for replacement options. 3D cell culture models represent the intestine better than 2D cell culture models do due to the presence of the different cell types, cell-cell interactions and cell-extracellular matrix interactions.

We generated intestinal organoids from adult stem cells retrieved from the crypts of the proximal and distal colon of healthy pigs, obtained from the slaughterhouse. These organoids contain all cell types found in the colon reflecting the in vivo situation. Induction of colitis in vitro was performed by treatment with inflammatory compounds on organoid derived monolayers which was confirmed via gene expression analysis. With this model, we aim to investigate the possible regenerative effect of the ERBB receptor family members to understand not only the underlying effects but provide also mechanisms for future therapies.

Therefore, our porcine 3D cell culture model shows not only an in vitro replacement to study colitis but also paves the way for replacing animal experiments where possible resulting in an overall reduction of the use of animal models in colitis research.

Abstract Number: 47

Poster

IN SILICO PREDICTION OF MYCOTOXIN PERMEABILITY THROUGH LIPOPOLYSACCHARIDE MEMBRANES

Nuša Matjašec[1 2], Giorgia Del Favero[1], Christian Schröder[2]

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During an infection cells are exposed to a wide variety of signals. Xenobiotics, as for example foodborne mycotoxins, can modulate the inflammatory processes, which can reduce the immune response from the host and potentially increase sensitivity towards pathogens. Zooming in on the infection site at molecular level, one less understood aspect is the interaction between chemicals and bacterial lipid membranes, which can influence the availability of the toxins with a potential "masking effect". Here, we developed an in silico model to simulate the mycotoxin interactions with bacterial membranes through molecular dynamics simulations. Our model recreates the outer lipopolysaccharide membrane of the gram-negative E. Coli, which is known for its protective function due to the oligosaccharide (OS) chains. Those membrane models were used to study the penetration, permeability behavior and residence time of selected mycotoxins such as alternariol, deoxynivalenol and tenuazonic acid. To highlight the importance of the OS region, simulations were also performed with a simplified lipid only membrane model. The results confirm the protective role of the OS, as mycotoxin penetration into the membranes drastically decreased upon addition of the sugar moiety for all selected ligands. Overall, this study presents a novel animal free bacterial membrane model that enhances the mechanistic understanding and prediction of mycotoxin absorption behavior, with applicability to other ligands.

Abstract Number: 53

Poster

INFECTION-DRIVEN CCR7 PROGRAMMING DIRECTS DENDRITIC CELL MIGRATION IN AN IMMUNOCOMPETENT HUMAN 3D SARS-COV-2 AIRWAY MODEL

Sophie Ann Erckert[1], Wilfried Posch[1]

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Human immunocompetent 3D airway models offer powerful, animal free systems to study respiratory virus–host interactions and support the implementation of the 3R principles. How dendritic cell (DC) positioning within the airway epithelium modulates early SARS CoV 2 control remains poorly understood. Using a primary human bronchial epithelial air–liquid interface model co cultured with monocyte derived DCs, we analyzed DC recruitment, CCR7 dependent migration programming, and antiviral activity during SARS CoV 2 infection. Viral exposure markedly increased active DC migration into the epithelial compartment. Interaction with differentiated airway tissue alone induced CCR7 expression in DCs, indicating that epithelial cues initiate migratory reprogramming without strong inflammatory signals. Basolateral supplementation with the CCR7 ligands CCL19 and CCL21 promoted DC egress, confirming chemokine guided trafficking. Notably, retention of DCs within the epithelium correlated with reduced viral RNA and infectious SARS CoV 2, consistent with enhanced local antigen uptake and antiviral mediator production while DCs reside in the tissue. These findings highlight DC positioning as a regulator of early infection outcomes and underscore immunocompetent human airway models as physiologically relevant, animal free approaches that contribute to Replacement and Reduction in respiratory virus research in accordance with the 3R principles.

Abstract Number: 55

Poster

TINY GUTS, BIG IMPACT: FUNCTIONAL ASSESSMENT OF BACTERIAL TOXICITY USING CANINE INTESTINAL ORGANOIDS

Kristina Schmidhofer[1] [2], Georg Csukovich[3], Alexandro Rodriguez Rojas[2], Ehling-Schulz Monika[1]

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Organoids have emerged as powerful tools to model organ physiology and host–pathogen interactions in vitro. In the field of food safety, organoids represent an innovative platform for evaluating microbial risks beyond classical taxonomy-based diagnostics. Conventional systems, such as Caco-2 cell lines, are widely used but have notable limitations in replicating the complexity of native tissue. Moreover, current systems often fail to distinguish between hazardous and non-hazardous bacterial strains, particularly when assessing toxicity.

Organoids, by mimicking the in vivo environment more realistically, yield more physiologically relevant results and may enable new interpretations of microbial pathogenicity, opening new possibilities for more accurate risk assessment.

Adult stem cell-derived canine intestinal organoids and organoid-derived monolayers (ODMs) are used to evaluate the cytotoxic effects of bacterial supernatants and toxins. These experiments reveal strain-specific toxicity profiles and provide mechanistic insight into bacterial pathogenesis at the epithelial interface. As humans and dogs share key features of intestinal structure and susceptibility to bacterial infections, data from canine organoids offer translational value.

By replacing redundant in vivo infection experiments and enabling more targeted follow-up studies, this in vitro platform supports more efficient and ethical use of resources, contributes to the 3Rs, and strengthens the One Health framework.

Abstract Number: 66

Poster

THE STUDY OF HOST-PATHOGEN INTERACTIONS OF CHLAMYDIA IN TRANSWELL-CULTURED PORCINE OVIDUCT EPITHELIAL CELLS

Juan Bernardo Odasso[1], Leonie Bettin[1], Babette Fletemeyer[2], Christine Unterweger[3], Doris Wilflingseder[2], Tobias Käser[1]

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Chlamydia suis (Cs) is the primary chlamydial species naturally infecting pigs and is associated with reproductive disorders, conjunctivitis, and enteric and respiratory infections. In addition to its veterinary importance, the highly similar Chlamydia trachomatis (Ct) is the leading cause of bacterial sexually transmitted infections and reproductive complications in women worldwide.

We not only aim to better study and understand Cs and Ct pathogenesis, but also to align with the 3R principles of replacement, refinement, and reduction in animal research.

This project establishes a long-term in vitro model using primary porcine oviduct epithelial cells (pOECs) left from slaughter house animals, cultured in a 3D air–liquid interface (ALI) transwell system.

As shown by histological and immunofluorescence staining, this approach promotes cell differentiation into a polarized epithelium consisting of mucin-secreting and ciliated cells. This epithelium established a consistent and high transepithelial electrical resistance (TEER)), closely mimicking in vivo conditions. It has also been successfully infected with Cs as shown by both live and fluorescence imaging. This cell culture system is currently being used to determine Cs influence on barrier epithelial integrity by TEER. A better understanding of these local host-pathogen interactions will also support the development of therapeutics and vaccines to improve both veterinary and human reproductive health.

Abstract Number: 94

Poster

THE ERBB RECEPTOR SYSTEM AS NOVEL THERAPEUTIC TARGET IN COLITIS

Katharina Zednik[1], Laura Geyer[2], Janina Burk[3], Franziska Dengler[4], Maik Dahlhoff[1]

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[4] Universität Hohenheim, Department of Livestock Tissue Metabolism, Institute of Animal Science, Stuttgart, Germany

Colitis is a severe inflammatory disease of the large intestine with diverse etiologies and a high unmet need for therapies that promote epithelial regeneration. Increasing evidence highlights ERBB receptor signaling, particularly via EGFR and ERBB4, as a key regulator of intestinal epithelial repair, identifying these pathways as promising therapeutic targets. To investigate the roles of EGFR and ERBB4 in colitis-associated regeneration, genetically modified mouse models lacking EGFR or ERBB4, as well as a transgenic mouse line with ubiquitous overexpression of the EGFR/ERBB4 agonist betacellulin (BTC), were generated and analyzed in experimental colitis. Transgenic BTC mice exhibited significantly attenuated colitis compared to controls. In line with the 3Rs, animal experiments were reduced by establishing colon organoids derived from the genetically modified mouse lines for downstream analyses. Notably, organoids derived from EGFR knockout mice failed to sustain growth, underscoring the essential role of EGFR signaling in epithelial maintenance. In contrast, ERBB4 knockout–derived organoids showed increased size, suggesting a distinct regulatory function of ERBB4. Together, these findings demonstrate that ERBB receptor signaling is crucial not only for colonic epithelial maintenance but also for colon organoid generation. ERBB-based organoid models provide a valuable platform for future studies on ERBB signaling in colonic diseases, including colitis and colorectal cancer.

Abstract Number: 160

Poster

ESTABLISHMENT OF A PHYSIOLOGICALLY RELEVANT, SPECIES-MATCHED 3D TRANSWELL MODEL TO STUDY CHLAMYDIA TRACHOMATIS INFECTION IN PORCINE OVIDUCT EPITHELIUM

Johanna Maier[1], Juan Bernardo Odasso[1], Leonie Bettin[1], Gerlinde Hofstetter[1], Doris Wilflingseder[2], Tobias Käser[1]

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[2] Ignaz Semmelweis Institute, Interuniversity Institute for Infection Research, University of Veterinary Medicine Vienna, Vienna, Austria

Chlamydia trachomatis is the most prevalent bacterial sexually transmitted pathogen and a major cause of reproductive tract disease in women. In vitro infection models depend on physiologically relevant epithelial systems, whose predictive value hinges on the extracellular matrix.

Collagen is widely used, yet most commercial sources are rat-tail derived, requiring dedicated animal sacrifice and creating a species mismatch for porcine models. Here, we establish and validate a protocol to isolate native type IV collagen from porcine eyes obtained as slaughterhouse by-products. Because pigs are bred for food production, this enables recovery of high-quality biomaterials without additional animal use, directly advancing the 3R principles. Species-matched collagen supports more physiologically relevant porcine cell culture. Type IV collagen was isolated by enzymatic/biochemical purification and verified by Western blot for purity and integrity. We compared it with type I collagen from porcine skin and commercial rat-tail collagen. Primary porcine oviduct epithelial cells (pOECs) were cultured on these substrates and characterized by immunofluorescence and histology. We assessed adhesion, epithelial morphology, and early differentiation markers to evaluate the impact of collagen origin and subtype.

Our data show that slaughterhouse-derived porcine collagens provide a robust, biologically relevant matrix for primary epithelial culture, eliminating the need for collagen sourced from animals sacrificed for research. This practical, scalable strategy refines experimental models, enhances translational relevance, and reduces animal use in biomedical research.

NAMS & 3RS IN ONCOLOGY

Abstract Number: 174

Presentation

ADVANCED PATIENT-DERIVED TUMOR ORGANOIDS TO STUDY THE CROSSTALK OF CELLS IN THE TUMOR MICROENVIRONMENT

Gerda Egger[1]

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Patient-derived tumor organoids (PDTOs) have emerged as powerful three-dimensional models that recapitulate the genetic landscape, architecture, and heterogeneity of different cancer entities. However, conventional PDTOs are primarily composed of epithelial tumor cells and lack critical components of the tumor microenvironment (TME), which plays a central role in tumor progression, immune evasion, and therapy response.

Using colorectal cancer PDTOs we recently demonstrated that incorporation of patient-matched fibroblasts enhances physiological relevance, promoting tumor cell heterogeneity and enabling reciprocal crosstalk that mirrors in vivo tissue organization. Notably, cancer-associated fibroblasts secrete a range of growth factors and cytokines that support PDTO growth in the absence of exogenous niche factors, thereby providing a more physiological tumor model compared to conventional stem cell–like culture conditions.

Building on this concept, we established advanced multicellular CRC organoid models integrating peripheral blood mononuclear cell (PBMC)-derived macrophages. Our system enables reconstruction of both normal and tumor-like microenvironments by combining normal organoids with normal fibroblasts and macrophages, or tumoroids with cancer-associated fibroblasts and macrophages. These models provide a versatile platform to dissect cellular interactions and immune-stromal crosstalk in CRC, offering new opportunities for mechanistic studies and therapeutic testing.

Abstract Number: 151

Presentation

VASCULARIZED TISSUE MICROARRAYS: A GENUINE HIGH-THROUGHPUT APPRAOCH TO DRUG SCREENING IN ADVANCED TUMOR MICROENVIRONMENT MODELS.

Katarzyna Rojek[1], Paulina Musolf[1], Jan Guzowski[1]

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Despite significant developments in endothelial-cell (EC) manipulation techniques, a proper in vitro model of a functional microvasculature with controlled local interconnectivity under well-defined global architecture is still lacking. Here, we report the generation of multi-scale vascular networks via manipulation of tens of sprouting EC ‘seeds’. We exploit magnetic patterning to assemble EC-coated superparamagnetic microbeads into ordered arrays and establish effective growth rules governing the development of interconnectivity and directionality of the networks depending on the applied seed-seed spacing.

We show that EC-seed-based approach offers a range of advantages over more conventional vascular tissue engineering techniques including: (i) expedited sprouting, (ii) spatial control over interconnections, (iii) reduction in cell consumption by even >100x, and (iv) native high-throughput format. We demonstrate that the microvascular arrays co-cultured with cancer cells can efficiently serve as a high-throughput platform for phenotypic screening of various anti-angiogenic compounds. Our model allows to faithfully emulate cancer-type specific morphology of the capillary networks and study their response to treatments.

Overall, we propose a uniquely precise and reproducible vascular-microtissue engineering tool with applications, e.g., in angiogenesis research, phenotypic drug screening and possible extension to immune-microenvironment engineering and organ-on-chip formats.

Abstract Number: 154

Presentation

3D-BIOPRINTED TUMOUR-ON-CHIP MODELS THAT MIMIC THE EFFECTS OF DRUGS AND TUMOUR METASTASIS IN VITRO.

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For decades, animal experiments were considered the gold standard for testing drugs and novel therapeutic approaches in tumour biology, despite significant physiological differences between humans and animals being the main reason why most drugs fail in clinical trials. To address this issue, we are developing microvascularised human tumour tissue models that are bioprinted into laser-manufactured fluidic chip devices. This approach combines the advantages of perfused fluidic chips with those of structured bioprinted tissue equivalents. We demonstrate that blood capillaries can be grown to form dense networks around tumour spheres in order to test the efficacy of potential anti-angiogenic substances. Tumour metastasis can be visualised 'online' and dissected into different stages, from intravasation and extravasation to the invasion of local or distant microtissues. In ovarian cancer, the critical metastatic step is the adhesion of floating cancer spheroids to the peritoneum. We investigate this process directly using a biofabricated mesothelium-on-a-chip together with cancer spheroids derived from ovarian cancer cell lines or patients' ascites. These models assess the direct impact of the tumour microenvironment on drug sensitivity, enabling different tissues to be combined on one chip for medium-throughput, high-content drug screening or validation applications. This provides a valid, human tissue-based alternative to the use of experimental animals in cancer research.

Abstract Number: 65

Presentation

MODELING NUCLEAR DEFORMATION AND DNA DAMAGE USING 2D AND 3D CELL CULTURES

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In vivo models have traditionally dominated oncology research, yet new approach methodologies (NAMs) provide alternatives that support the 3Rs principles while improving mechanistic insight. Advanced in vitro models enable the investigation of processes that are difficult to isolate in vivo, such as nuclear mechanotransduction. [1]

Nuclear deformation can occur through extracellular forces (e.g. confined migration), causing DNA damage, supporting carcinogenesis. In this work, we investigate the movement of intracellular organelles as an overlooked source of forces acting on the nucleus, potentially supporting genetic instability. We recently demonstrated that treatment of SKOV3 ovarian and T24 bladder cancer cells with bafilomycin A1 induced a repositioning of the mitochondria toward the perinuclear region. This shift was associated with nuclear deformation, altered Lamin A/C organization, and DNA damage.

Building on these findings obtained in 2D models, we are further developing 3D spheroid systems to bridge the gap to the in vivo tumor architecture. Cells in 3D environments experience spatially heterogeneous physical forces, including compression in the spheroid core and tension at the periphery. These models provide a physiologically relevant, animal-free platform to study how mechanical stress influences nuclear integrity, mechanotransduction, and chemoresistance, advancing oncology research while adhering to the the 3Rs.

[1Jobst et al.(2025) doi:10.1016/j.isci.2025.112955

Abstract Number: 87

Presentation

HUMAN-RELEVANT 3D ALI LUNG MODELS FOR BALF BIOMARKER DISCOVERY IN NSCLC

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Non-small cell lung cancer (NSCLC) remains a leading cause of cancer-related mortality worldwide, largely due to late-stage diagnosis. There is an urgent need for clinically relevant diagnostic biomarkers. Bronchoalveolar lavage fluid (BALF) reflects the lung tumour microenvironment and has strong diagnostic potential in NSCLC; however, limited access to patient-derived BALF samples and the lack of human-relevant BALF animal models hamper biomarker discovery. To address this, our project investigated the use of 3D air-liquid interface (ALI) in vitro models of NSCLC to generate BALF-like samples and identify soluble and extracellular vesicle (EV)-associated biomarkers. Mono- and co-culture ALI cancer models from human NSCLC cells, alongside three healthy primary alveolar ALI models, were used. BALF-like samples were harvested from the models via apical saline washes, mimicking clinical procedure, and then analysed for protein content, oncogenic proteins, and EV-associated surface markers. Cancer ALI models showed significant upregulation of osteopontin and VEGF, previously reported in clinical literature as present in patient-derived BALF, as well as a potentially novel candidates. Key findings were validated using ELISAs. Our results highlight the value of 3D ALI models as human-relevant, non-animal platforms for translational biomarker discovery and open the way to BALF-based diagnostics for NSCLC.

Abstract Number: 171

Presentation

YOUCELL: PERSONALIZED TUMOR CELL LINE MODELS

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The establishment of clinically relevant models remains a central challenge in translational cancer research. Within the YouCell platform, we generate personalized tumor cell line models by isolating and expanding primary tumor cells together with cancer-associated fibroblasts (CAFs), enabling improved representation of the tumor microenvironment. A key strength of our approach is the rapid processing of fresh surgical specimens and seamless transition from tissue acquisition to model establishment. This embedded setting allows characterization using the same diagnostic standards applied to patient samples, ensuring high biological fidelity. We established a diverse panel of tumor models derived from sarcoma, pancreatic, breast, and ovarian carcinomas. For each model, master and working cell banks were generated, accompanied by comprehensive quality control, including characterization and stability assessment. These patient-derived systems enable diverse downstream applications. As a proof of concept, we present drug response profiling using a matched panel derived from a patient's primary tumor, metastases, CAFs, and skin fibroblasts, highlighting tumor–stroma interactions. The YouCell platform provides a scalable, reproducible framework for personalized tumor modeling and investigation of tumor biology, including genetic and epigenetic characteristics.

Abstract Number: 34

Poster

A HUMAN 3D BONE MARROW MODEL FOR IMPROVING NK CELL IMMUNOTHERAPY IN ACUTE MYELOID LEUKEMIA

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Acute myeloid leukemia (AML) is a heterogeneous hematologic malignancy defined by the accumulation of immature blasts in the bone marrow (BM), leading to high relapse rates and limited treatment options. Disease progression and resistance to therapy are driven by the BM microenvironment, which promotes leukemic survival and suppresses anti-tumor immunity. Natural killer (NK) cells, which eliminate cancer cells without prior exposure, have shown promise as an innovative immunotherapeutic approach. However, their impact remains limited due to the immunosuppressive BM niche, which includes mesenchymal stromal cells (MSCs), favoring leukemic survival and impairing NK cell activity. Standard 2D in vitro models poorly reflect the spatial and cellular complexity of this niche. To address this, we developed a 3D culture model comprising human MSCs, AML cells, and NK cells, where all three cell types remained proliferative and functional. However, NK cell activity was significantly impaired in the presence of MSCs, mirroring in vivo observations. This suggests that our system could resemble the human BM niche. Using this model for transcriptome and secretome analyses will allow us to identify key regulatory pathways that drive immune suppression and leukemic cell survival. Overall, our approach bridges the gap between traditional in vitro and in vivo research, offering an animal-free alternative to support the development of NK cell-based immunotherapies for AML.

Abstract Number: 40

Poster

AUTOLOGOUS 3D TUMOR-CAF CO-CULTURE MODELS AS ADVANCED IN VITRO SYSTEMS TO STUDY THE TUMOR MICROENVIRONMENT

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Accurate cancer modelling is limited when tumour cells are studied alone, as the absence of stromal components like cancer-associated fibroblasts (CAFs) fails to reflect patient tumour behaviour. Tumour–stroma interactions are therefore mostly studied in animal models, despite translational and ethical limitations. To reduce animal use, we established autologous tumour models combining patient-derived tumour cells with CAFs from the same tissue. The panel includes rare sarcomas (clear cell sarcoma, CIC–DUX4 sarcoma, dedifferentiated chondrosarcoma) and epithelial cancers (cholangiocarcinoma, pancreatic and breast cancer). Scaffold-free 3D spheroids were generated in ultra-low attachment plates, enabling physiologically relevant interactions without exogenous matrices. CAF identity was confirmed by α-SMA and FAP expression. Co-culture altered spheroid morphology and increased structural stability and tumour–stroma interactions were entity-specific, with

spatial organisation resembling parental tissue architecture. In cholangiocarcinoma models, CAF co-culture induced changes in proteomic and secretory profiles, reflecting functional tumour-CAF crosstalk.

Collectively, these autologous 3D co-culture systems provide a robust and ethically responsible in vitro alternative to animal models, supporting the 3R principles while enabling clinically relevant investigation of tumour–stroma interactions and therapeutic responses.

Abstract Number: 50

Poster

IMPLEMENTING SILICA-BASED MESOPOROUS NANOPARTICLES TO MIMIC PARTICULATE MATTER AND BACTERIA-SHAPE IN AN INTESTINAL MODEL

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Disruption of the intestinal barrier is increasingly recognized as a driver of chronic inflammation and colorectal cancer, as barrier failure enhances epithelial exposure to microbial and dietary carcinogens. Mimicking the gut's complexity, understanding intestinal cell interactions with materials of different shapes can inform drug delivery design, nanostructure-based toxicity models, and the assessment of foodborne nano- and micro-fragments from degraded contact materials or contaminants. Building on recent studies implementing spherical, rod-like, and virus-like morphologies in the Caco-2/HT29-MTX-E12 model, a novel system was developed to probe intestinal barrier function at the nanoscale using mesoporous silica nanorods (SiO_2-NPs). Functionalization of spherical SiO_2-NPs modulated their mobility through mucus and cell interactions depending on hydrophobicity and charge. Here, rod-shaped mesoporous SiO_2-NPs (small rods: 35 × 160 nm; bacteria-like rods: 200 × 450 nm) were synthesized and chemically modified to tailor mucus passage and cell interactions. Surface methylation or phosphonation markedly altered cell-cell contact and penetration depth, showing that NPs-cell interactions depend on both geometry and surface chemistry, with implications for barrier pathology, carcinogenesis, and the behavior of particulate contaminants such as micro- and nanoplastics.

Abstract Number: 56

Poster

A 3D-BIOPRINTED MESOTHELIUM-ON-CHIP TO UNCOVER OVARIAN CANCER INVASION BEHAVIOR

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Ovarian cancer (OvCa) is the fifth-common cause of cancer death and has a 5-year survival rate of about 50%. One reason for its high mortality rate is the occurrence of multicellular aggregates in the ascites of more than 50% of OvCa patients. These spheroids are most likely a cause for metastasis and relapse. Xenograft, syngeneic and genetically engineered mouse models have been described for OvCa, but none of them allow a direct investigation of the human situation of OvCa-spheroid invasion into mesothelium.

Using 3D-bioprinting, we developed a unique in vitro system for studying OvCa-spheroid mesothelium interaction, the Mesothelium-on-Chip. Read-out methods like fluorescence imaging with measuring the area of invasion and OvCa sphere size, as well as surface profiling were implemented for this model. We investigated the invasion behavior of different OvCa cell lines and were able to show inhibition of their invasion potential with different treatment options.

This model will replace animal experiments in OvCa research and allow us to better understand the role of mesothelium and tumor spheroids in OvCa disease progression. It will assist to predict patient's therapy response using ascites-derived multicellular aggregates and has unmet potential for testing novel therapeutics in a human, organotypic system to improve patient-tailored therapy.

Abstract Number: 83

Poster

PROGRESS MONITORING OF HUMAN MICROTUMORS ON CONTROLLED MICROENVIRONMENTS

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Tumor research still relies heavily on mouse experiments, including therapeutic xenograft models. In this poster we advance the 3Rs by developing human cell–based in vitro alternatives that enable continuous, non-destructive readout of time-dependent biology—tumor growth and progression, invasion/metastatic spreading, and responses to drugs or immunotherapies—while recreating key microenvironmental cues.

We combine label-free real-time impedance spectroscopy with microstructured chip technology. Sensor-patterned surfaces, micrometer-scale cell chambers and microfluidic elements provide controlled perfusion, compartmentalization and tunable biochemical/mechanical conditions for 2D cell layers and 3D tumor spheroids.

With a first microstructured chiw we demonstrates on-chip monitoring of human tumor spheroids (and in future: of patient-derived microtumors). After adding anticancer drugs or cytotoxic T cells, characteristic impedance signatures report therapy efficacy and kinetics in real time, reducing the need for animal studies. In a second study we present our multichannel microfluidic chip with multiple microcompartments and integrated electrodes designed to establish a vascularized dermis–epidermis model under controlled flow.

We conclude with future prospects and current limitations of chip-based in vitro systems as scalable, reproducible replacements for animal experiments in oncology and beyond.

Abstract Number: 101

Poster

A NOVEL GLIOBLASTOMA CELL BANK FOR MODELLING INTER-PATIENT HETEROGENEITY

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Glioblastoma (GBM) is a highly aggressive and heterogeneous brain tumour, for which pre-clinical studies still rely on long-established and extensively passaged in vitro models that insufficiently reflect patient variability.

To address this limitation, we established a patient-derived GBM cell line biobank, using a standardized workflow for tissue processing and culture. Sixteen tumours were collected after surgery and long-term cultures were established from eight patient samples, including a relapse and a rare post-mortem spinal metastasis. Each cell line was validated against its parental tumour, showing strong concordance in copy number variation and mutation profiles.

Phenotypic and molecular analyses revealed marked inter-patient heterogeneity, with distinct morphologies and variable expression of glial and stem markers (GFAP, CD133). Multi-omics profiling highlighted further diversity. MiRNA sequencing identified recurrent signatures across cell lines, including miR-21-5p, miR-221/222-3p, miR-93-5p and miR-125b-5p, all linked to oncogenic traits, alongside miRNAs such as miR-31-5p, miR-99a-5p and miR-224-5p, highly expressed only in subsets of cells, suggesting patient-specific regulatory programs. Metabolomics and drug screening confirmed functional heterogeneity and variable therapeutic responses.

This novel GBM biobank provides a representative pre-clinical platform for studying tumour heterogeneity, biomarker discovery, and personalized therapeutic strategies.

SPOTLIGHT ON REFINEMENT: GENETICALLY MODIFIED ANIMALS - ASPECTS OF GENERATION AND USE + PANEL DISCUSSION

Abstract Number: 165

Presentation

GRUNDLAGE ZUR BELASTUNGSBEURTEILUNG GENETISCH VERÄNDERTER NAGERLINIEN IN ÖSTERREICHISCHEN EINRICHTUNGEN ZUR ZUCHT UND HALTUNG VON VERSUCHSTIEREN.

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Dieses Dokument präsentiert eine standardisierte und wissenschaftlich fundierte Grundlage zur Belastungsbeurteilung genetisch veränderter Nagerlinien in österreichischen Versuchstiereinrichtungen. Ziel ist es, eine einheitliche, nachvollziehbare und vergleichbare Bewertung des Tierwohls in österreichischen Einrichtungen zu ermöglichen und gleichzeitig Antragstellende sowie Behörden bei Genehmigungs- und Kontrollprozessen zu unterstützen.

Die Leitlinie definiert rechtliche Rahmenbedingungen, zentrale Begriffe und Kriterien zur Einstufung des Schweregrades genetisch veränderter Linien sowie Anforderungen an deren Etablierung und Haltung. Besonderes Augenmerk liegt auf der systematischen Erfassung von Belastungen anhand standardisierter Beobachtungskategorien, einschließlich klinischer, verhaltensbezogener und umweltbedingter Parameter. Zudem werden klare Vorgaben zur Stichprobengröße, zu Beobachtungszeitpunkten über den Lebensverlauf dargelegt.

Das Dokument differenziert zwischen belasteten und nicht-belasteten Linien und berücksichtigt Sonderfälle wie konditionale, induzierbare und Reporterlinien. Es trägt damit zur Verfeinerung (Refinement) und Reduktion (Reduction) bei und unterstützt eine evidenzbasierte, transparente Belastungsbewertung. Insgesamt stellt es ein praxisorientiertes Instrument dar, das die Harmonisierung von Tierwohlstandards und die Qualität wissenschaftlicher Forschung in Österreich nachhaltig fördern soll.

SPOTLIGHT ON REPLACEMENT: ANIMAL-FREE & GREEN LAB CONCEPTS FOR THE FUTURE

Abstract Number: 139

Presentation

ADVANCES IN PLACENTAL BIOMATERIALS FOR VASCULAR SCAFFOLDS, DRUG DELIVERY SYSTEMS, AND 3D BIOPRINTING

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Human placental tissues represent a unique and ethically accessible source of extracellular matrix (ECM) for the development of advanced biomaterials. We report recent advances in human placenta–derived ECM (hpECM) platforms for vascular scaffolds, drug delivery systems, and 3D bioprinting applications, supporting the implementation of 3R principles in translational research.

Decellularised placental arteries and veins are processed into off-the-shelf small-diameter vascular scaffolds with preserved microarchitecture and tunable mechanical properties. In parallel, solubilised hpECM is formulated into injectable hydrogels (2–8 mg/mL) and printable bioinks for extrusion-based 3D bioprinting. These human-derived matrices provide a tissue-specific microenvironment promoting endothelialisation, vascular network formation, and controlled therapeutic loading.

Compared to conventional animal-derived matrices, hpECM biomaterials enhance human relevance and reduce dependence on xenogeneic products. Applications include perfusable in vitro vascular models, regenerative implants, and localised drug delivery systems. By integrating decellularisation, biofabrication, and functional testing, placental biomaterials offer a versatile and scalable platform for refining preclinical research and reducing animal experimentation.

Human tissues were obtained with informed consent and ethical approval. The authors declare no conflicts of interest.

Abstract Number: 23

Presentation

A NOVEL 3D-PRINTED MOUSE MODEL FOR SURGICALTRAINING

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Simulation-based training can replace or reduce live animals and cadavers in early-stage rodent surgery education while supporting competency requirements.

We developed a 1:1 scale 3D-printed “3R Mouse” midline laparotomy simulator with replaceable synthetic abdominal wall layers. In a multicenter validation study (5 academic centers in Europe/USA), 44 participants (29 novice, 15 expert) performed standardized incision and closure tasks across repeated iterations. Construct validity was assessed via task times and double-blind photo-based quality scoring; face and content validity via post-use surveys.

Novices significantly improved speed and suture quality with repetition and, by iteration 5, reached performance comparable to experts’ first attempt. Experts remained faster and achieved higher quality scores, confirming discrimination by experience level. Both groups rated anatomic fidelity and overall realism highly and judged the simulator very/ extremely useful for instrument handling and basic suturing; all participants would recommend it.

The 3R Mouse demonstrates face, content and construct validity for foundational rodent surgical training and competency assessment, offering a scalable, animal-free approach that can reduce and partially replace animal use in training.

Abstract Number: 52

Presentation

CONTINUOUS MONITORING OF OXYGEN, PH, GLUCOSE AND LACTATE IN MICROPHYSIOLOGICAL SYSTEMS WITH INTEGRATED OPTICAL SENSORS

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Constant monitoring of culture parameters like oxygen, pH, and glucose is needed to ensure physiological conditions in microfluidic cell cultures or Organ-on-a-Chips (OoC). Furthermore, the measurement of these parameters can give valuable information on the viability and metabolic state of the cultured cells. Despite the large improvement of organ-on-chip systems in the recent years there is a lack in the development of efficient in-situ readout techniques for micro physiological systems and organ-on-chips. Analysis of organ-on-chips is challenging due to the small dimensions and sample volumes. Currently, this is mainly achieved by optical and fluorescence microscopy using stains and labels, which can be used for one single measurement. In recent years our group has established integration of sensors for the key metabolic and culture condition parameters oxygen, pH, glucose and lactate. Miniaturized luminescent sensors spots are integrated into various microfluidic cell and tissue culture devices. The sensor spots in a size from 500 to 800 µm and be read-out with a commercially available instrument. We present various examples of microfluidic cell chips with integrated sensors and performance data, demonstrating the monitoring of metabolism in WAT (white adipose tissue) and liver organ models.

Abstract Number: 84

Presentation

R3ACT: A REDOX AND ANTIOXIDANT CAPACITY TRACKER FOR ANIMAL-FREE DRUG SCREENING IN HUMAN KERATINOCYTES

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R3ACT (Redox & Antioxidant Capacity Tracker) is an animal-free, oxygen-managed live-cell screening workflow designed to capture early redox and antioxidant signalling before loss of viability. The platform uses genetically engineered HaCaT keratinocytes maintained at 5 kPa O_2 and integrates oxygen-regulated compound exposure with high-content imaging. This setup is motivated by the fact that many in vitro assays are still run in room air (≈18–20 kPa O_2), while many tissues operate at much lower oxygen (≈1–8 kPa O_2), which can shift oxidative-stress readouts and complicate redox hazard interpretation. R3ACT tracks H_2O_2 dynamics using the HyPer7.2 biosensor targeted to the cytosol and mitochondria, quantifies Nrf2 signalling with the POINTER reporter, and monitors viability in parallel to interpret responses within non-lethal windows. Dose–response outputs are condensed into standardized response scores via a dedicated analysis workflow, and automated deep-learning single-cell quality control is used to improve data reliability and hit selection. Method performance was benchmarked with perfluorooctanoic acid, polystyrene nanoplastics, zinc oxide, and salicylic acid; reproducibility was tested in two independent runs, and a compound was called a hit only if it met the criteria in both repeats. Overall, R3ACT provides a scalable oxygen-controlled NAM workflow for redox-sensitization screening, supporting applications in cosmetics assessment and early drug development.

Abstract Number: 128

Presentation

SHARING IS CARING: REPLACING LIVE ANIMAL MODELS THROUGH SYSTEMATIC USE OF LARGE-ANIMAL EX-VIVO TISSUES

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The use of animal tissue offers strong potential for implementing Replacement, as many research questions can be addressed ex vivo. While abattoir material often lacks standardization, tissue from laboratory animals provides a well documented, high quality resource.

The project “ShaRing is CaRing” establishes a structured, legally compliant process for collecting, storing, and distributing animal by products from mice, rats, rabbits, pigs, sheep, and frogs. When using rodent tissue, the digital platform “3R blackboard”, linked to an animal management software, enables efficient sharing while ensuring compliance with material transfer agreements.

Since 2022, a standardized workflow has been implemented with authorities, including mandatory request forms, veterinary checks, defined transport routes and an updated registry ensuring full traceability. Trained staff collect and prepare tissue according to user needs (fresh, preserved, or frozen).

From 2022–2025, 1024 organs from 482 animals were distributed to 78 research groups. On average, nearly four projects were supplied per animal, substantially reducing additional animal use. Numerous studies in surgery, pharmacology, and anatomy were performed entirely ex vivo. Training formats also benefited, enabling surgical practice on animal bodies and reducing burden in later in vivo studies.

Overall, our data show that coordinated use of animal tissues advances Replacement while improving resource efficiency and scientific validity.

Abstract Number: 11

Presentation

ADVANCED MODELING OF LCHADD/VLCADD WITH 3D-BIOPRINTING AND INDUCED-PLURIPOTENT STEM CELL TECHNOLOGY

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Background: Long-chain-3-hydroxy-acyl-CoA-dehydrogenase-deficiency (LCHADD) and Very-long-chain-acyl-CoA-dehydrogenase-deficiency (VLCADD) are rare disorders of the oxidation of long-chain fatty acids that often cause severe cardiac complications, including cardiomyopathy and arrhythmia.

Methods: We analyzed mitochondrial morphology in β oxidation defective fibroblasts treated with various rescuers using live cell fluorescence microscopy. A 3D-bioprinted, vascularized tissue model with healthy and patient-derived fibroblasts was established to mimic physiological conditions. Patient fibroblasts were reprogrammed into iPSCs and differentiated into cardiomyocytes to model cardiac complications.

Findings: Patient fibroblasts showed significant mitochondrial alterations and elevated intracellular ROS levels driven by NOX2. Mitochondrial network integrity was restored by several rescuing compounds. In 3D tissue constructs, patient cells impaired vessel formation, which which also improved upon treatment with rescuing compounds. iPSC-derived cells exhibited a profibrotic phenotype with osteogenic and chondrogenic, but lacking adipogenic, differentiation and increased stress fibers. Cardiomyocytes showed morphological abnormalities and altered beating compared to controls.

Interpretation: These models provide insights into mitochondrial dysfunction and ROS imbalance in LCHADD/VLCADD and enable investigation of cardiac complications and patient specific treatment responses.

Abstract Number: 44

Presentation

ADVANCING REPLACEMENTS: A BIOREACTOR PLATFORM FOR IN VITRO HYPERTENSION MODELING AND DRUG SCREENING

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Hypertension is a primary global risk factor, yet a significant gap exists between static cell cultures and complex in vivo models. This study bridges this gap culturing HUVECs within IVTech bioreactors platform that provides a realistic in vitro surrogate for hypertensive vessel walls. The system integrates a pressure-actuation device (LivePa) and adjustable flow controls (LiveFlow), enabling precise decoupling of biochemical stimuli (Angiotensin II) from mechanical intraluminal pressure. To validate the system's predictive power without laboratory animals, we conducted a systematic review following PRISMA guidelines, analyzing inflammatory activation in Spontaneously Hypertensive Rats (SHR) from 1985-2025. This 40-year benchmark confirmed that our in vitro signatures—IL-8 secretion and NF-κB/p38 phosphorylation—mirrored pathological trends in hypertensive animal vessels. Testing molecule IPU 3l demonstrated the platform's resolution, identifying context-dependent effects (ET-1 and inflammatory reduction after hypertensive stimuli) that animal models often fail to isolate. By aligning in vitro results with decades of SHR research, this platform provides a validated alternative for drug testing, supporting the 3Rs framework through Replacement of animal models with high-fidelity data. Support: Alan and Helene Goldberg program (CAAT Grant #2023-02); Global 3Rs Award 2022. Ref Raschi et al. 2026 (10.3389/fphys.2025.1724932)

Abstract Number: 19

Poster

REPLACEMENT OF EXPERIMENTAL ANIMALS BY 3D PRINTED MODELS FOR INTRAVENTRICULAR INJECTION TRAINING

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Stereotaxic surgery is a minimal invasive technique commonly used for injections or electrode implantation in rodents. Whereas in human medicine it is supported by imaging methods, in animal experiments it mostly relies on the experience of the conductor. Therefore, extensive training is required. In the case of using rats and mice for practice, repeated use of heads and brains is not possible, which increases the demand of animals, and incorrect injection may lead to animal suffering, which is a major ethical concern. These could be overcome by using non-animal models (NAMs).

Three rats were euthanized and injected with a contrast agent, after which they were scanned using micro–computed tomography. The resulting datasets were segmented in 3D Slicer to generate anatomical models. These models were subsequently 3D printed, and brain tissue was mimicked with silicone.

The finalized model can be mounted in a stereotaxic frame, allowing burr holes to be drilled at anatomically accurate locations. A catheter can then be inserted, and correct placement can be verified through fluid aspiration.

This model provides an ethical and low-cost training tool allowing repeated practice without the use of live animals. Consequently, it is consistent with the 3Rs, replacing animals in training with a NAM and reducing overall animal use.

Abstract Number: 31

Poster

POPULATION-SPECIFIC TRANSCRIPTOMIC AND MOLECULAR INSIGHTS INTO PRPF31-ASSOCIATED RETINITIS PIGMENTOSA USING RETINAL ORGANOIDS

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Retinitis Pigmentosa (RP) caused by PRPF31 mutations leads to progressive photoreceptor loss and blindness. This study integrated human-induced pluripotent stem cell (hiPSC)-derived retinal organoids with single-cell RNA sequencing (scRNASeq) to identify population-specific transcriptomic changes and pathogenic mechanisms. Patient iPSCs (PRPF31 c.1115_1125 del11) and control were differentiated into organoids and analyzed at early (d85) and late (d285) developmental stages.

scRNASeq revealed significant disruptions across retinal populations. Mutant photoreceptors showed downregulated phototransduction and oxidative phosphorylation pathways, with progressive degeneration by d285. Müller glia exhibited reactive gliosis and inflammatory stress, while ganglion cells showed dysregulated axon guidance. Temporal analysis highlighted early dysfunction preceding late-stage loss. Findings were validated via RT-qPCR and immunofluorescence, which confirmed reduced phototransduction markers and increased gliosis. Functional multielectrode array (MEA) recordings further demonstrated diminished electrophysiological activity in mutant organoids.

By aligning with 3R principles, this human-derived model provides a robust alternative to animal studies. These results uncover specific mitochondrial and inflammatory drivers of PRPF31-associated RP, providing a foundation for targeted therapies to mitigate retinal neurodegeneration.

Abstract Number: 48

Poster

ALTERNATIVE METHOD FOR MURINE PDAC MODELS

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Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer and has a 5-year survival rate of only about 13%. In vivo mouse models are commonly used to simulate PDAC for cancer research and drug testing. 2D cell culture models have not been a successful alternative, because pancreatic acinar cells are difficult to culture.

The aim of our research is to develop a suitable alternative to animal models for PDAC research, by using 3D bioprinting. Bioprinting is a 3D printing method where cells and biomaterials, called bioinks, are used to create a 3D tissue or 3D cell culture environment. By bioprinting 3D models with murine pancreatic cells, an artificial exocrine pancreas is created, that could be used to investigate the mechanisms of PDAC or any other pancreatic disorder in more detail in vitro.

Our first experiments show promising results that pancreatic acinar cells survive longer in 3D bioprinted models and do not transdifferentiate into ductal cells, like they do in 2D culture. These 3D bioprinted cancer models could be perfect systems to replace animal experiments with an in vitro model and fulfil the 3R idea to refine, reduce and replace animal experiments.

Abstract Number: 61

Poster

A 3D IN VITRO BONE MARROW MODEL TO REPLACE ANIMAL-BASED STUDIES OF HEMATOPOIETIC NICHE INTERACTIONS

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Background: Direct cell–cell and cell–matrix interactions regulate the maintenance, migration, and differentiation of hematopoietic progenitors and shape their behavior in the presence of leukemic cells. To replace animal-based approaches, a controllable and imaging-compatible bone marrow model is required to study these interactions under physiologically relevant conditions.

Methods: We established a 3D bone marrow model based on a gently crosslinking hydrogel matrix that allows spatially defined zones and culture periods of two weeks. A murine lineage-negative, Sca-1^{+}, c-Kit^{+} hematopoietic progenitor cell line (HPC^{LSK}), supported by MS-5 stromal cells, was used as a sensitive readout to assess viability, 3D migration, depth distribution, and early differentiation markers.

Scope: The model is designed to recapitulate key niche cues and to enable quantitative analysis of direct interactions between normal progenitors and leukemic cells. Following optimization with HPC^{LSK} cells, the system will be extended with AML cell lines to enable quantitative analysis of direct interactions between normal progenitors and leukemic cells, including migration-based niche preferences.

Conclusion: This 3D bone marrow model preserves essential HSC-associated properties, enables quantitative live analyses in a defined microenvironment, and provides a scalable, low–animal-use platform to study leukemia-driven niche alterations and directly compare HPCLSK and AML cells within the same architectural context.

Abstract Number: 79

Poster

BEYOND BOVINE: HIV-1 INFECTION IN HUMAN PLATELET LYSATE-BASED MONOCYTE-DERIVED DENDRITIC CELLS

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Fetal calf serum (FCS) is the gold standard for monocyte-derived dendritic cell (moDCs) cultivation, but it adds unknown and unquantified components that limit reproducibility and physiological relevance in in vitro immune models. Therefore, we substituted FCS with human platelet lysate (hPL) for moDC generation to study HIV-1 infection. Primary CD14^{+}

monocytes were differentiated into CD11c⁺CD209⁺ moDCs using hPL and compared phenotypically and functionally to FCS-based DCs. Using hPL monocytes differentiated efficiently to moDCs, which showed increased adherence and higher survival post-infection compared to FCS-derived moDCs. Treatment of moDCs with complement-opsonized HIV-1 (HIV-C) from both culture conditions showed characteristic expression of activation markers and loss of CD11c, indicating maturation in response to HIV-C application. Quantitative analyses using p24 ELISA and confocal imaging illustrated that complement opsonization enhances viral uptake compared to non-opsonized HIV-1 in all culture conditions. Importantly, hPL conditions supported DC viability and functionality in a fully human, animal component-free setting. This animal-free in vitro platform reduces experimental variability and provides a physiologically relevant system for studying DCs for their responses to HIV-1, with an extensive potential for future investigations into different stimulants, such as other viruses.

Abstract Number: 90

Poster

ADVANCING RHEUMATOID ARTHRITIS RESEARCH WITH A BIOMECHANICAL SYNOVIUM-ON-A-CHIP MODEL

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Existing models for rheumatoid arthritis (RA), including mostly animal-based and 2D in vitro systems, fail to replicate the complexity of human RA pathophysiology fully. Therefore, more physiologically relevant in vitro RA models are urgently needed to understand disease mechanisms better and enable ethical, human-based research. This project addresses these limitations by developing a fully human, patient-derived synovium-on-a-chip model that integrates co-cultures of synovial fibroblasts, immune cells, and endothelial cells under biomechanical loading.

One of the primary aims is to establish animal-free cultivation conditions optimized for each cell type, along with a triple co-culture system. We will test alternatives to fetal calf serum and fetal bovine serum using both 2D and 3D culture systems, including Panexin CD, placenta extract, human serum and human platelet lysate. Additionally, various hydrogels (Peptimatrix, HyStem®, VitroGel® COL High) will be tested to find the most suitable for replication in vivo conditions, applying different biomechanical loading parameters, such as physiological strain and fluid shear stress, closely mimicking the mechanical microenvironment of the human synovium.

This approach aims to advance human-based RA modeling while supporting the reduction of animal use and improving the physiological relevance of in vitro disease studies.

Abstract Number: 91

Poster

PHOTORESPONSIVE HYDROGELS FOR MECHANIC STIMULATION IN LIGAMENT CELL-ON-CHIP APPLICATIONS

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Ligamentopathies and tendinopathies are multifactorial disorders involving inflammation and extracellular matrix degradation driven by abnormal mechanical loading, yet their cellular mechanisms remain poorly understood, in part because most in vitro models fail to reproduce the native mechanical microenvironment.

This project addresses this limitation by developing a cell-on-chip system enabling controlled mechano-stimulation of multiple cell-laden hydrogel constructs (microactuators) and simultaneous real-time readouts with integrated biosensors.

Microactuators were fabricated by stop-flow lithography (SFL) using Poly(N-isopropylacrylamide) (PNIPAM) with gold nanoparticles and Polyethylene Glycol Diacrylate (PEGDA). In a second SFL step, a biocompatible hydrogel containing ligamentocytes was polymerized into the PNIPAM/PEGDA construct. Characterization included image-based actuation analysis, COMSOL-based laser heating simulations, and cell morphology and viability assessment. Surface plasmon resonance (SPR) was used to measure MMP3 and IL6 for on-chip biosensing.

SFL enabled reproducible fabrication of U-shaped microactuators with defined geometry for mechanical stimulation. Viability assays showed over 90% ligamentocyte survival in methacrylate collagen I, hyaluronic acid methacrylate, and human platelet lysate methacrylate, confirming biocompatibility. Preliminary SPR detected IL6 and MMP3, supporting integration of label-free molecular readouts with mechanical actuation.

Abstract Number: 104

Poster

THE ROLE OF HYDROSTATIC PRESSURE IN BIOMIMETIC CELL CULTURE SYSTEMS

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Air-interfacing tissues such as airway and mucosal epithelia are primarily exposed to environmental toxicants via gases, aerosols, and particulate matter. In real-world settings, these exposures occur as complex, low-concentration mixtures such as urban smog and fine dust, with adverse effects emerging only after prolonged exposure and difficult to reproduce in vitro. Toxicological studies therefore still heavily rely on animal models or long-term clinical observations, highlighting the need for improved phase-specific exposure strategies in vitro.

To address the first limitation, a modular microfluidic, pressure-driven exposure platform is proposed to enable controlled application of aerosols, and particulate matter to air-interfacing tissues. Complementary in situ exposure to complex environmental pollutants is achieved via thermodesorption based enrichment.

The current system demonstrates sensor integration for monitoring temperature, relative humidity, gas concentration, pressure, and flow, enabling characterization of enrichment and transport. Initial operation of a self-regulating thermodesorption unit confirms the feasibility of controlled adsorption–desorption cycles and trace-gas handling.

The concept addresses a key methodological gap in in vitro toxicology by enabling relevant exposure of air-interfacing tissues to gases, aerosols, and particulate matter, improving assessment of phase-dependent effects while reducing reliance on animal inhalation studies.

Abstract Number: 113

Poster

MODELLING THE JUNCTION BETWEEN THE INTESTINAL EPITHELIUM AND THE ENTERIC NERVOUS SYSTEM USING HUMAN-DERIVED PHOTOPOLYMERISABLE HYDROGELS

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The enteric nervous system (ENS) is a neural network within the intestinal wall, linking the gut to the central nervous system and is increasingly recognised as a key player in gastrointestinal and neurological disorders. This underscores the need for advanced models to study these ENS–gut interactions in detail. Bioengineered, photopolymerisable hydrogels have shown the ability to recreate crucial biophysical and biochemical features of the gastrointestinal tissue matrix through spatially controlled light-based patterning and thereby present a key component of advanced cell culture models. Yet their integration into organ-on-chip (OoC) platforms remains limited, and existing systems often lack realistic tissue architecture, mechanics, and spatial heterogeneity. Here, we integrate a human-derived methacrylated hydrogel into a microfluidic device to generate biomimetic 3D architectures via photopatterning. Precise spatial control using photomasks reproduced villus–crypt-like structures and desired matrix heterogeneity. The platform was characterised for mechanical properties, molecular transport, and fluid dynamics. Intestinal epithelial cells and human enteric glial cells were used to assess the effects of gel architecture and matrix heterogeneity on cellular

phenotypes. This approach combines human-based photopolymerisable hydrogels with OoC technology to create a structurally complex, biologically relevant in vitro model to study ENS-gut interactions.

Abstract Number: 115

Poster

HUMAN PLATELET LYSATE AS A SERUM SUBSTITUTE FOR HUMAN CELL–BASED MODELS

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The FWF-funded REPLACE project aims to develop a fully human, animal-free tumorigenicity model as an alternative to xenogeneic mouse models. The project focuses on establishing an immunocompetent, vascularized skin tumour model on a microfluidic chip by integrating epidermal, dermal, and vascular compartments derived exclusively from human cells. Nutrient and signalling exchange are mediated via micro-permeable barriers, while tumour progression is monitored using integrated bioimpedance sensing. This approach provides a standardized and ethically acceptable platform for translationally relevant tumorigenicity testing and personalized on-chip cancer models without animal use.

To ensure fully animal-free culture conditions, human platelet lysate (hPL) was implemented as a universal, human-based serum substitute. Human melanoma cell lines, primary fibroblasts, and vascular endothelial cells were successfully adapted from FBS-containing to hPL-based culture conditions. Cell morphology, viability, proliferation, phenotypic marker expression, and genetic identity were maintained. hPL was produced at University Clinic for Blood Group Serology and Transfusion Medicine using a buffy coat–based platelet pooling strategy combined with leukocyte depletion, ensuring high standardization and reduced donor variability.

Overall, these results validate hPL as a robust serum substitute and support its use in reproducible, fully human in vitro tumour models for cancer research.

Abstract Number: 124

Poster

A FEASIBILITY STUDY ON AMBIENT CONDITION SHIPMENT OF 3D CULTURED CELL LINES IN MICROFLUIDIC MULTIARRAY DEVICE

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The shipment of cell lines is commonly performed either in a viable state or under cryopreserved conditions. Both approaches rely on specialized and costly courier services and are frequently associated with transport-related stress, resulting in reduced cell viability.[3Three-dimensional (3D) multicellular spheroids exhibit increased robustness against chemical and mechanical stress compared to conventional two-dimensional cultures, making them attractive candidates for resilient cell transport.[1,2Here, we present a microfluidic platform for the formation, cultivation, and transport of spheroids derived from adherent cell lines to reduce transport associated costs and cellular stress. [4The device allows for in situ spheroid formation and maintenance in a pH-controlled environment during transport, showing stable metabolic activity over a 72-hour period under ambient conditions. Following transport, the spheroids are enzymatically dissociated and successfully re-plated in two-dimensional culture. Gene expression analysis targeting pathways associated with cellular stress, survival, apoptosis, and cell–matrix interaction evaluates the post-transport cell performance to investigate the feasibility of spheroid-based microfluidic devices as a practical alternative to current cell shipment methods.

[110.1152/physiol.00036.2016
[210.1038/nrc.2015.13
[310.1089/bio.2023.0100
[410.1002/advs.202004856

Abstract Number: 134

Poster

THE RTGILL-W1 CELL LINE AS A SPECIES-RELEVANT, IN-VITRO MODEL FOR ICHTHYOTOXICITY TESTING

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To date, aquatic toxicity of environmental contaminants and natural toxins is predominantly assessed using whole-organism bioassays, ranging from brine shrimp to fish larvae and adult fish. While widely accepted, these assays are time-consuming, costly, and associated with substantial animal use and welfare concerns. In addition, acute and sub-lethal fish toxicity tests provide very limited mechanistic insight, yielding only relative toxicity without identifying specific modes of action.

In line with the 3R principles, fish cell lines offer a species-relevant, cost-effective, and ethically preferable alternative that enables mechanistic investigations and the assessment of multiple endpoints. The Organisation for Economic Co-operation and Development (OECD) Fish Toxicity Testing Framework promotes such alternative approaches, and the OECD Test Guideline 249 (2021) describes an acute toxicity assay using the rainbow trout gill cell line RTgill-W1.

In our study, we used the RTgill-W1 cell line to investigate the modes of action of selected ichthyotoxins produced by microalgae. Our results highlight the scientific value and regulatory potential of these in-vitro, species-relevant systems for mechanistic toxicity assessment, toxicity screening, and their role in advancing 3R strategies in aquatic toxicology.

Abstract Number: 153

Poster

HUMAN PLACENTA ECM BIOINKS TO REPLACE ANIMAL MATRICES IN 3D CARDIAC MODELS

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Biomaterials of decellularized human placental extracellular matrix (dhpECM) offer high translational potential due to reduction of animal-derived materials, protein composition, and accessibility. This study aims to identify placental compartment–specific properties for selecting tissue-mimetic matrices for cardiac in vitro models.

Hydrogels from four placental compartments - amnion, basal plate, chorion, and umbilical cord were fabricated. Proteomics analysis was used for predicting tissue specificity. Cell interaction in vitro was evaluated using human cardiac fibroblasts. Rheology quantified mechanical properties, and printability was validated on a custom in-gel extrusion bioprinter.

Proteomics revealed distinct tissue compositions and matrisomes. Spearman correlation ranked the umbilical cord matrices closest to native cardiac tissue. Enrichment confirmed the umbilical cord as a candidate, as cardiac terms were significantly enriched in this dataset. Preliminary gene expression trends corroborated in silico rankings, confirming umbilical cord-derived matrices moderating fibroblast activation markers while controlling matrix expression. Thermogelation (G' > G") and shear thinning were characterized, allowing for stable 3D printed constructs.

Umbilical cord-derived ECM bioinks showed highest biochemical fidelity to cardiac tissue and printable mechanics. By leveraging a human ECM source, the approach supports human-relevant 3D cardiac culture over animal-derived matrices.

SPOTLIGHT ON REFINEMENT: ANALGESIA & ANESTHESIA + PANEL DISCUSSION

Abstract Number: 82

Presentation

ANALGESIC DRUG TREATMENT VIA DRINKING WATER: A WATERTIGHT APPROACH?

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Due to its non-invasive nature, voluntary oral self-administration via drinking water (d.w.) may represent a favorable administration route for analgesic drugs in rodents. However, limited acceptance and variable fluid intake might hamper the achievement of adequate plasma levels. This study assessed oral acceptance, plasma concentration-time profiles, and tolerability of standard analgesics administered via d.w. over five days. Female and male rats (n=159) received carprofen, metamizole, buprenorphine, or tramadol, while mice (n=252) were treated with the same drugs plus meloxicam and butorphanol. Target dosages corresponded to maximum recommended doses based on current expert information and common practice in laboratory animal analgesia. Mice generally accepted drug-containing d.w. well, usually resulting in stable plasma levels within 12-24 h of treatment initiation. Meloxicam required sweetening, showed marked circadian variation in plasma levels, and caused moderate side effects. Rats showed dislike of tramadol and almost completely refused metamizole. Carprofen was well tolerated, whereas buprenorphine and tramadol induced notable behavioral side effects, including pica. Overall, administration via d.w. requires close monitoring to ensure constant target dose intake, and some analgesics cannot be recommended in the tested doses due to adverse effects or poor intake. Rats appeared more susceptible to side effects and taste-related aversion than mice.

Abstract Number: 80

Presentation

MULTIMODAL ANALGESIA FOR POST-OPERATIVE PAIN IN MICE AND RATS: A SYSTEMATIC REVIEW IN PROGRESS

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Clinical studies in humans show superiority of multimodal over single agent analgesia for postoperative pain; however, in laboratory rodents the evidence remains unclear. To synthesize results from existing data on multimodal analgesia in mice and rats, a systematic review (SR) protocol was created based on the SYRCLE guidelines and preregistered at OSF Registries. PubMed, WoS, and CABI Digital Library were searched, and 3,085 articles were screened. Article selection was performed in two phases (title/abstract; full text) with predefined exclusion criteria by two independent reviewers per article, resulting in 63 articles for data extraction and analysis. Results show more articles in rats (n=42) than mice (n=21), where both sexes were used only in 4/42 and 7/21 articles. In both species, plantar incision was the most frequently used surgical model, and in mice, NSAID/opioid co-administration was the most investigated multimodal approach. Across studies, the analgesic regimens varied considerably in drug choice and dosing regimens, and the use of multiple complementary pain surrogate markers was infrequent. Overall, the current body of evidence is heterogeneous and methodologically inconsistent, which limits recommendations for evidence-based multimodal analgesia. Controlled preclinical multi-center studies are urgently needed to improve laboratory rodent pain management.

Abstract Number: 38

Presentation

A NOVEL TIME-CONTINUOUS MRI METHOD TO QUANTIFY IN-VIVO BROWN ADIPOSE TISSUE THERMAL POWER: REFINEMENT AT A GLANCE

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Since the rediscovery of brown adipose tissue (BAT) in adults, positron emission tomography has been widely used to assess BAT activation via glucose uptake. In rodents, metabolic cages are commonly employed to estimate thermogenesis from whole-body energy expenditure. However, neither approach directly reflects the intrinsic thermogenic capacity of BAT, underscoring the need for more precise in-vivo measurement techniques.

We developed a time-continuous MRI protocol that combines proton MR spectroscopy with a MRI blood perfusion sequence to monitor BAT activity. BAT temperature was derived from the water-proton resonance frequency, while the spectra additionally provided quantitative information on neutral lipid content without additional acquisition time. Heart rate and rectal temperature were recorded simultaneously. BAT thermal power was calculated from BAT temperature and perfusion in conjunction with rectal temperature. The protocol was evaluated in cold-adapted and thermoneutral housed mice using adrenergic stimulation with norepinephrine or CL316,243.

We quantified BAT blood flow, temperature, lipid content, and thermal power in a time-resolved manner within a single, integrated experiment. Adrenergic stimulation induced distinct temporal changes in BAT physiological parameters and clearly discriminated between the two stimulants. Together, these data provide a direct, quantitative, and comprehensive picture of BAT activation in vivo.

Abstract Number: 86

Presentation

PERCEPTION BIAS IN ASSESSING RODENT WELFARE DURING EUTHANASIA AND ANAESTHESIA

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Although euthanasia is routine in animal experimentation, its welfare implications remain debated, and performing euthanasia can be ethically challenging and linked to moral distress. Beyond perceived unjustified reasons for killing (e.g. surplus), uncertainty about animal suffering contributes to ethical uncertainty in decision-making. Prior work demonstrated that welfare assessments of mice can be influenced by contextual framing, with identical videos interpreted differently when presented as anesthesia/ euthanasia. Such perception bias may compromise objective welfare assessment.

In a mixed-methods design (eye-tracking, think-aloud interviews, and repeated welfare ratings), 35 professionals viewed 4 short videos depicting rodent anesthesia or euthanasia in a randomized cross-over design. After each video, participants rated welfare on four 0–9 Likert items assessing fear/escape behavior, respiratory, and overall distress. Quantitative data were analysed using linear mixed-effects models; qualitative data were thematically coded.

Euthanasia was rated as more distressing than anesthesia. Crucially, anesthesia videos framed as euthanasia were rated as more distressing than the same videos correctly framed, indicating perception bias. Eye-tracking showed increased attention to respiratory-related regions under euthanasia framing. Interviews revealed ambiguity in rating escape and fear behaviors and difficulties assessing grimace indicators independently of respiratory distress.

Abstract Number: 148

Presentation

HOW DO WE MANAGE PAIN IN MICE? A SURVEY OF ANALGESIA IN ONGOING EXPERIMENTS

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Effective pain management is central to Refinement in mouse studies, yet routine analgesia and monitoring practices across facilities are incompletely documented. We surveyed current facility practice to map analgesia strategies and pain assessment before, during and after procedures and to identify barriers to implementation. An anonymous 34-item online questionnaire (RepRefRed Society/Austrian 3R Center for BMFWF) launched 22 Sep 2025; an interim analysis from January included complete datasets from respondents (n=81 of 125 total respondents). Respondents were predominantly from academia (76.5%) in Austria/Germany (63%/37%); 77.8% had at least weekly animal contact. Non-surgical invasive procedures were most common (48.1%). Pain was assessed mainly by behavioural observation (88.9%) and published scoring schemes (81.5%). NSAIDs dominated (80.2%), most often given subcutaneously or via drinking water. Pre-emptive analgesia was routine in 46.9% and multimodal regimens in 50% (n=80). Main barriers were time constraints (24.7%), limited application routine (21.0%), drug availability (19.8%), staffing (17.3%) and lack of 24-h monitoring. These interim results provide a practice snapshot and identify targets for training, facility support and national 3R priorities.

SPOTLIGHT ON 3RS & NAMS: INNOVATIVE MODELS & METHODS

Abstract Number: 163

Presentation

I LIKE TO MOVE IT': ROBOTIC MICROSYSTEMS FOR MUSCULOSKELETAL DISEASE MODELLING.“TITEL: „„I LIKE TO MOVE IT': ROBOTIC MICROSYSTEMS FOR MUSCULOSKELETAL DISEASE MODELLING

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Musculoskeletal diseases are a leading cause of disability. Progress in therapy development is hindered by preclinical models that do not capture the broad spectrum of human-specific physiology and mechanobiology. Conventional in vitro systems lack dynamic mechanical cues, while animal models often fail to translate to human outcomes. This gap underscores an urgent need for more predictive, human-relevant screening platforms. Robotic microsystems, combining precise actuation, sensing, and control at the microscale, offer a powerful solution by recreating physiologically relevant forces and tissue interactions. These systems enable deeper insight into disease mechanisms and hold promise for accelerating the development of effective therapies. The current talk aims to highlight how rapid prototyping, soft robotics, and a bit of good old LEGO can be utilized to develop advanced human-relevant tissue platforms for basic and advanced 3R focused sciences.

Abstract Number: 69

Presentation

LOST IN TRANSLATION: MULTI-SPECIES TRANSCRIPTOMICS REVEAL MOLECULAR DIVERGENCE IN CHONDROCYTE INFLAMMATION

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The failure of animal models to predict human disease mechanisms and drug responses has been a major barrier to developing curative osteoarthritis (OA) therapies, yet reliance on them persists. To inform evidence-based model selection, we systematically compared inflammatory responses of articular chondrocytes from six species (human, horse, sheep, rabbit, rat, mouse) under standardized IL-1β and TNF-α stimulation in 2D and 3D cultures. Readouts included viability, proliferation, wound healing, and transcriptomics via 3'RNA-seq. Marked interspecies differences were observed in morphology, cytokine sensitivity, and inflammatory gene induction. Only two genes (SOD2, NFKBIA) were conserved across species. Canonical inflammatory and OA-associated pathways active in human cells were absent, attenuated, or divergent in animal models. These findings demonstrate fundamental molecular discordance between animal models and human OA, rendering continued animal experimentation scientifically unreliable for OA therapeutic target discovery, drug development and efficacy testing. Continued reliance on such models in OA research risks systematic translational failure while imposing a substantial and avoidable animal burden. Mechanistically faithful human-cell-based in vitro systems, including advanced 3D models, therefore represent a scientifically necessary and ethically aligned alternative that directly advances Replacement and Reduction while improving translational relevance.

Abstract Number: 105

Presentation

ADVANCING IN VITRO MODELING OF THE INTESTINAL BARRIER FOR CELIAC DISEASE RESEARCH

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The intestinal epithelial barrier is a central regulator of host-environment interactions and is impaired in multiple diseases. Among these, celiac disease, a gluten-sensitive enteropathy, affects at least 1% of the global population with a 1.5-fold higher prevalence in women. Although disease mechanisms are well understood, a strict gluten-free diet remains the only therapeutic option, indicating limitations of currently existing preclinical models for translational drug or food-derived biomolecule screening. Hence, we first aim to establish a scalable human intestinal barrier model based on a Caco-2/HT29-MTX E12 co-culture to study prolamin-mediated effects. After 21 days of differentiation, the model forms a functional epithelial barrier exhibiting polarization, mucus production, barrier integrity, and digestive enzyme expression. Exposure to -gliadin p31-43 did not impair barrier integrity but induced a significant increase in IL-8 secretion as an inflammatory reponse. However, since cell line-based models fail to replicate tissue complexity and disease-specific hallmarks, in a next step patient-derived organoids are being established and integrated into a microphysiological platform. This multi-model framework enables comparative analysis reflecting human physiology across increasing levels of biological complexity to improve the understanding of the effects of cereal-derived biomolecules on the intestinal barrier.

Abstract Number: 112

Presentation

VALIDATION OF NEW APPROACH METHODOLOGIES IN GLIOBLASTOMA ORGANOIDS USING INTERSTITIAL FLUID PROFILING AND CEREBRAL OPEN FLOW MICROPERFUSION (COFM

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Background and Purpose: Animal models remain the main preclinical tool for studying glioblastoma biology and therapy response, despite limited translational relevance and growing ethical pressure to reduce animal use. New approach methodologies (NAMs), such as glioblastoma organoids, offer human-relevant alternatives but require validation against in vivo reference models. Interstitial fluid (ISF) is a suitable matrix for such comparative validation.

Methods: Cerebral open flow microperfusion (cOFM) enabled atraumatic access to ISF in orthotopic human glioblastoma xenografts. Tumor cells were implanted via the cOFM probe, allowing tumor development at the probe–tissue interface while preserving blood–brain barrier integrity. ISF, cerebrospinal fluid, and plasma were analyzed using HILIC-HRMS-based metabolomics.

Results: Nearly 400 metabolites were detected, with 281 identified in ISF. ISF showed the most pronounced tumor-associated metabolic alterations (>30% significantly changed vs. controls), compared with cerebrospinal fluid (7%). Pathway analysis indicated dysregulation of lipid metabolism, ABC transporter activity, central carbon metabolism, and amino acid pathways linked to redox homeostasis.

Conclusion: cOFM-based ISF profiling captures tumor-specific metabolic features of glioblastoma and provides a standardized in vivo reference to support validation of NAMs such as organoids, enhancing translational relevance while contributing to the refinement of animal experimentation.

Abstract Number: 63

Presentation

DEVELOPMENT OF AN IMMUNOCOMPETENT 3D BIOPRINTED MELANOMA -ON-CHIP MODEL

Alexeja Kleiter[1], *Patrizia Stoitzner*[2], *Judith Hagenbuchner*[1], *Michael Ausserlechner*[1]

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Melanoma is one of the most aggressive forms of skin cancer. Understanding tumor–immune interactions is crucial for the development of effective therapeutics. However, translating findings from murine models to human patients remains a significant challenge. To address this, we developed a human immunocompetent 3D bioprinted melanoma-on-chip model using a custom-designed microfluidic chips. Human fibroblasts (HFF) and keratinocytes (HaCaT) were embedded in a GelMA/collagen bioink containing Pluronic F127 channels for nutrient supply and cultured at the air–liquid interface for two weeks. The resulting skin construct exhibited proper keratinocyte differentiation (AE1-3, cytokeratin 1/10 positive), Ki67 expression restricted to basal layers, and elongated fibroblast morphology. The 3D bioprinted skin remained viable for up to three weeks.

MugMel2 melanoma spheroids were subsequently embedded within the skin constructs and cultured for three weeks. Spheroid growth was monitored by laser-scanning microscopy, and immunohistological analyses confirmed S100 and Ki67 expression while preserving the integrity of the surrounding tissue. Overall, this 3D melanoma-on-chip platform represents a novel and physiologically relevant preclinical model for studying tumor–immune interactions and evaluating therapy strategies in vitro.

Abstract Number: 60

Presentation

REPRODUCTIVE OUTCOME AND STRESS RESPONSES AFTER SURGICAL OR NON-SURGICAL EMBRYO TRANSFER IN MICE – A REFINEMENT STUDY.

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Embryo transfer (ET) is pivotal in assisted reproductive technologies for laboratory mice, enabling rederivation, revitalization, and establishment of transgenic lines. Switching from surgical ET (SET) to non-surgical methods would reduce the burden for the animals and therefore be in line with the 3Rs. This study aimed to compare SET with non-surgical ET methods (using the NSET or TCET device) in terms of reproductive success rates and animal welfare (stress measured as fecal corticosterone metabolites, CM). Blastocysts for ET were generated either by in vitro fertilization and subsequent culture, or ex vivo, cultured from flushed zygotes or morulae. SET and non-surgical methods showed comparable implantation rates (76-85%) for ex vivo embryos, with NSET without anesthesia offering the simplest, least invasive approach. Independent of the transfer method, birth rates were significantly higher for ex vivo (55-67%) compared to in vitro generated embryos (4-22%). In addition, blastocysts from flushed morulae yielded superior outcomes over blastocysts from flushed zygotes (birth rate: 73% vs. 19%), highlighting culture-related obstacles. Individual stress levels (rise of fecal CM) were highest after SET compared to non-surgical methods. In conclusion, non-surgical ET methods demonstrate equivalent success rates to SET for ex vivo embryos while reducing stress. However, in vitro embryo generation remains less efficient, with reduced viability linked to extended culture durations.

NAMS & 3RS IN CLINICAL TRANSLATION

Abstract Number: 26

Presentation

MECHANOINFLAMMATORY MODULATION OF GUT-DERIVED LPS RESPONSES IN A HUMAN SYNOVIAL BIOCHIP

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Synovial inflammation drives osteoarthritis (OA) and rheumatoid arthritis (RA). Gut-derived lipopolysaccharides (LPS) contribute to joint inflammation, yet their interaction with joint-relevant mechanical cues is poorly understood. We developed a fully human, animal-free synovial biochip to study mechanoinflammatory modulation of LPS responses.

Primary fibroblast-like synoviocytes (FLS) from OA patients and non-OA donors were cultured in 3D hydrogels under static conditions or pneumatically actuated joint-like compression. Structurally distinct LPS variants (10 ng/mL) were applied. In parallel, bone marrow-derived mesenchymal stromal cells (MSC) were cultured in 3D matrices and exposed to short-term pneumatic activation with or without LPS. Inflammatory and matrix-related gene expression (IL6, CXCL8, MMP1, MMP3, COX2, PRG4) was analysed by qPCR and normalized to static controls.

LPS induced donor-heterogeneous inflammatory responses in FLS with LPS type-dependent differences. Mechanical stimulation amplified inflammatory responses in FLS but attenuated LPS-induced pro-inflammatory and catabolic gene expression in MSC, demonstrating cell-type-specific mechanoinflammatory regulation along the gut–joint axis.

Abstract Number: 67

Presentation

DEVELOPMENT OF A DYNAMIC THREE DIMENSIONAL IN-VITRO EQUINE TENDON MODEL.

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Tendinopathy is a chronic, irreversible degenerative disease linked to tendon overuse, and most current invitro models don't mimic its biomechanical environment.

This study develops a scalable dynamic 3D invitro equine tendon model using a 3D printed uniaxial straining device and custom made silicone culture strips to apply controlled physiological and pathological strain to multiple tendon like constructs simultaneously.

Healthy equine tenocytes (n=4)(1000cells/µl) were embedded in 40% collagen1 (PureCol, Sigma), 60% medium (89%DMEM, 10%FCS, 1%PenStrep and cultured in DMEM-filled wells, promoting self-organization into 3D tendon-like structures (3DTs) by contraction of the hydrogel between the anchors. After one week, 3DTs were cultured statically or exposed to a dynamic strain regimen (5% or 10%; 8 × 1 h cycles/day with 2 h breaks). Cell viability (FDA/PI staining), cellular alignment along the long axis (histology) and adequate nutrient diffusion (computational fluid dynamics) was confirmed.

mRNA expression (qPCR) of tenocyte extracellular matrix, inflammation and biomechanical markers and next generation sequencing revealed statistically significant differences between static and strained tendons after 1week, highlighting the role of mechanical stimulation in tendon phenotype maintenance.

This dynamic 3D-tendon model offers a scalable, biologically relevant platform for studying tendon mechanobiology and disease, with applications in drug testing and regenerative medicine.

Abstract Number: 131

Presentation

TRANSLATIONAL BIOIMAGING: FUNCTIONAL BARCODING OF DYNAMIC SIGNALING PROCESSES AND METABOLISM IN 2D & 3D MODELS

Sandra Burgstaller[1], Yusuf Erdogan[1], Ali Akyol[1], Anna Lischnig[1], Emrah Eroglu[2], Roland Malli[1]

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Understanding dynamic signaling processes and metabolism in alternative biomodels is essential for advancing the 3R principles in biomedical research. However, quantitative and multiparametric readouts in 2D and 3D systems such as iPSC derived cells and tissue models remain limited, particularly when real time activity and cellular history need to be captured. At the Medical University of Graz, the Core Facility Bioimaging is developing a Translational Bioimaging pipeline. This concept aims to integrate advanced microscopy, automated stimulation, and a broad range of genetically-encoded biosensors for scalable applications in 2D and 3D models. It builds on both established biosensors, and next generation recorders such as SPARK phase separating kinase sensors [1], SplitHaloTag integrators [2], and time-encoded intracellular ticker tape systems [3]. These tools are intended to provide multiparametric readouts of instantaneous signaling states, time integrated activity, and temporally resolved events. By combining multiplexed biosensor imaging with complementary microscopes and AI assisted data analysis, the Translational Bioimaging pipeline seeks to enable functional barcoding of cells, quantitative characterization of metabolic heterogeneity and high throughput workflows. This framework is intended to support predictive drug screening, patient specific disease modeling, and the development of robust and personalized in-vitro systems, thereby contributing to the 3R principles.

Abstract Number: 93

Presentation

HUMAN 3D AIRWAY MODELS AS DIAGNOSTIC TOOLS TO ASSESS RESPIRATORY PROTECTION

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Human immunocompetent 3D airway models provide a human based, animal free platform to evaluate respiratory protection after vaccination or infection in line with the 3R principles. Conventional neutralization assays rely on immortalized cell lines and quantify antibody mediated virus inhibition, but they do not reflect epithelial barrier function, tissue damage, or inflammatory responses. We used highly differentiated primary human airway epithelial air–liquid interface cultures infected with replication competent SARS CoV 2 variants and supplemented them with serum basolaterally and saliva apically. This system enabled parallel readouts of infectious virus production, transepithelial electrical resistance as a marker of tissue integrity, and local inflammatory activation. Samples with similar neutralization titers in standard cell lines showed divergent performance in the 3D tissue, where high salivary antibody activity associated with reduced viral replication, preserved barrier integrity, and dampened inflammatory signaling. These findings indicate that human 3D respiratory models provide more functionally relevant information on protection than conventional neutralization assays alone and may better identify individuals at risk, guide booster strategies, and reduce the need for animal challenge experiments.

Abstract Number: 14

Poster

PORCINE ADIPOSE SPHEROIDS - A SUITABLE TOOL TO ANALYZE OBESITY-RELATED PROTEINS

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Obesity is a growing global problem that reduces healthy life expectancy by almost 10 years due to associated comorbidities such as type 2 diabetes, cardiovascular disease and cancer. The drugs used to date that have been tested on animal models, often act systemically and are associated with a wide range of side effects. Therefore, it would be of great interest to identify mechanisms that specifically target adipocytes. Robustly established in vitro model systems can enable high-throughput screening and significantly reduce the number of animals used. One promising approach is the use of spheroids from porcine adipose tissue. Pigs are a particularly well-suited translational model organism in obesity research due to the comparable physiology of their digestive tract and fat distribution, as well as a similar expression of adipokines and regulators of lipid metabolism (e.g. PPARG). Tissue originating from meat production can be used to isolate porcine adipose derived stem cells. These cells are archived, genetically modified using CRISPR/Cas9 or RNAi as required, and finally differentiated into 3D-Models. By using different breeds with defined metabolic phenotypes, porcine spheroids allow population-relevant differences to be mapped. Compared to human primary cells, the better documentation of nutrition and genetics enables a systematic analysis of protein-dependent effects on lipogenic and lipolytic processes, for example after knockout of ERBB or LRIG proteins.

3RS & NAMS IN AQUATIC MODELS

Abstract Number: 164

Presentation

KEEPING ZEBRAFISH IN RESEARCH FACILITIES – FOCUS ON ENRICHMENT AS THE BASIS FOR RELEVANT AND RELIABLE RESEARCH

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The zebrafish is an established animal model for basic and preclinical research, and the number of zebrafish used in scientific studies continues to increase.

In recent years, there has been a growing focus on establishing standardized husbandry routines, including various forms of enrichment for zebrafish kept in laboratory environments. Stringent husbandry routines and a clear strategy for environmental enrichment are essential for obtaining relevant and reliable scientific data.

The zebrafish core facility at Karolinska Institutet serves more than 60 internal and external research groups as well as companies with validated experimental pipelines in basic, biomedical, and translational research. Since its establishment, the facility has placed strong emphasis on animal welfare and the standardization of both husbandry and experimental workflows.

In my presentation, I will describe the different environmental enrichment strategies that we have implemented in our facility. One of my main focuses will be the standardized structural enrichment developed in recent years, which has gained significant attention in the zebrafish community.

Abstract Number: 159

Presentation

DRUG SCREENING IN ZEBRAFISH LARVAE

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In search of new treatments drug screenings are integral part of the process, which usually starts with cell culture experiments. However, for defining effectiveness and safety, tests in living organisms have to be conducted. Zebrafish larvae are filling gap here between in vitro models and tests in mammalians. They offer several benefits: large clutches at low cost, complete organism, but fit into multi-well plates, small compounds for treatment are simply dispensed into water, they are transparent allowing for live imaging and before 120 hours post fertilization they are feeding only from their yolk. The development of zebrafish larvae is well described, so alterations in body maturation during drug treatment can also be used as toxicity read-out.

Xenograft models to study cancer treatments in mice are well established, however, we and others could also establish in zebrafish xenograft models. We set up an innovative platform to automatize key steps in the drug screening workflow with zebrafish larvae transplanted with various pediatric cancer cells using high-content imaging for monitoring the behavior of the tumor in a corporeal environment. We assessed small molecules that target specific cancer cell vulnerabilities and importantly effective combination treatments. This allows for guided pre-selection for only a small panel of potential drugs, which need to be tested in mammals eventually.

Abstract Number: 144

Presentation

NEEDS-BASED FEEDING IN DIFFERENT ZEBRAFISH FACILITIES

Verena Mayer[1], *Stefanie Kirchberger*[2], *Verena Ruprecht*[3], *Daniela Pollak*[4]

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[4] Institute of Molecular Pathology (IMP), Vienna, Austria

The feeding of zebrafish (Danio rerio) varies greatly between laboratory animal facilities, as there is no standardized diet, only general recommendations. In this presentation, we provide an insight into the feeding practices of different zebrafish facilities in Austria. We compare the types of administration, feeding frequencies, and quantities to highlight similarities and differences. The focus is on practical aspects of needs-based feeding and its importance for animal welfare and experimental quality. The aim is to provide suggestions for needs based feeding practices in zebrafish husbandry and to promote an exchange of experience between facilities.

Abstract Number: 109

Presentation

ADVANCING 3R STANDARDS FOR AMPHIBIAN MODELS IN BIOMEDICAL RESEARCH

Daniela Pollak[1]

[1], Vienna, Austria

Amphibians, particularly axolotls and frogs, are scientifically indispensable in some fields of biomedical research. However, the application of the 3Rs to these species is currently limited by a significant lack of evidence-based data. While mammalian welfare standards are well-established, the amphibian research community faces a shortage of 3R-relevant information, including species-specific analgesia, anaesthesia, and validated clinical indicators for pain and distress. Furthermore, reduction strategies such as cryopreservation remain largely unestablished.

This data deficit necessitates a clear mandate, and challenging opportunities for amphibia-targeted 3R strategies. We highlight ongoing efforts to address these gaps through practical welfare tools. Our approach integrates a framework for daily health checks and score sheets tailored to the unique physiology of the axolotl, refined housing and a specific breeding policy. We have also implemented standardized protocols for common health issues and a pathogen monitoring program.

While these steps represent progress, they underscore the need to further amphibia 3R efforts. Advancing this field requires a collaborative effort involving veterinarians, scientists, and care takers. By collecting empirical evidence, sharing best practice examples and targeted ressearch, we can move beyond anecdotal husbandry toward a validated framework supporting animal wellbeing and high-quality amphibian research.

Abstract Number: 74

Presentation

LIVE-CELL MICRONUCLEUS TESTING IN FISH CELLS FOR GENOTOXICITY ASSESSMENT

Magdalena Kainberger[1], *Petra Stahlschmidt-Allner*[2], *Verena Charwat*[1]

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The in vitro micronucleus (MN) test is a key method for identifying aneugenic and clastogenic substances. We refined a testing approach based on brain-derived, strictly epithelial growing fish stem cells to evaluate their suitability for advanced MN applications. These cells display distinct metabolic and embryonic features such as aryl hydrocarbon receptor expression. Variants expressing the histone marker H2B GFP enable automated kinetic live-cell imaging in a 96 well format, providing real-time visualization of chromosomal alterations and cell cycle dynamics. In addition, types of chromosomal instability such as nuclear buds, nucleoplasmic bridges, and karyorrhectic events can be detected directly in situ.

Using model compounds, we demonstrated reliable detection of both aneugens and clastogens in accordance with OECD Test Guideline 487.

Preliminary data indicate that the fish cell line exhibits metabolic competence. This suggests the potential use of these cells for testing pro-genotoxins without reliance on animal-derived S9 fractions for metabolic activation. Pending further validation, fish cell models may provide a mechanistically informative, animal-free alternative for genotoxicity testing in accordance with the 3R principles.

TRANSPARENCY & COMMUNICATION

Abstract Number: 166

Presentation

THE IMPORTANCE OF COMMUNICATING THE 3R'S PROGRESS AND REALITIES

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Remarkable advances in 3R-related research have been made since their conception almost 70 years ago and continue to be made at an ever-increasing pace. Available data underlines its impact on reducing the number of animals and their suffering in various domains. Internationally, strategies and roadmaps promise to further accelerate regulatory uptake of NAMs and phase-out of animal use in regulatory testing. These undoubted successes of the 3R principle are often neglected in public or political discourse.

At the same time, there is no indication that animal use will be fully dispensable in life-science research in the foreseeable future. Together, these different developments require careful and differentiated discussion within the scientific community, with policy makers, and with other interested parties and stakeholders of the public to enhance understanding, streamline regulation and avoid ethics dumping. Both, progress and realities, like knowledge gaps, are key messages for any meaningful debate.

Abstract Number: 17

Presentation

BUILDING BRIDGES THROUGH COMMUNICATION IN 3R RESEARCH

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Communication is an essential driver for implementing the 3R principle and for building trust in animal research across professional and social boundaries. Within our 3R Network in North Rhine-Westphalia, Germany, communication represents a core work area alongside science, networking, and education, enabling exchange, visibility, and uptake of 3R innovations.

For research community, we established practice-oriented communication formats such as the Paper of the Quarter, a contest for wider promotion of 3R related research from the regional scientific community. In parallel, our expert-curated website serves as a central hub providing resources, recommendations, and guidance to support 3R implementation. These activities are complemented by an active LinkedIn presence, facilitating dialog beyond institutional and national borders and reaching a rapidly growing international audience.

We developed a modular school program introducing pupils to animal research and 3R methods at increasing levels of depth, encouraging dialog with experts and peer-to-peer discussion. This program received a Best Poster Award at the Openness Conference in 2024. Additional outreach formats include panel discussions and participation in local science events, fostering transparency and exchange with the public. Current initiatives focus on evaluating public attitudes toward animal research and assessing the impact of our educational activities to inform evidence-based refinement and transferability.

Abstract Number: 54

Presentation

FROM INCONSISTENCY TO COHERENCE: INTRODUCING THE COMPOUND MODEL AS A PRAGMATIC SOLUTION FOR HARM-BENEFIT ANALYSIS

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[2] Columbia University, Institute of Comparative Medicine, New York, United States

The evaluation of project proposals using animals for scientific purposes is strictly regulated by EU Directive 2010/63/EU, introducing harm-benefit analysis (HBA) as one of the requirements. HBA's application in real-life review settings remains challenging, with recent studies identifying inconsistencies and the risks arising from these discrepancies, often attributed to the lack of feasible and practically useful models. This presentation therefore introduces a novel HBA method—the compound model (CM). The CM integrates robust components from existing guidance while omitting elements that risk introducing inconsistency into HBA implementation. The CM addresses all of the HBA's constituting components, referred to as domains, namely harm, outcome, benefit, justification, and ethical considerations. However, (i) ethical considerations are intentionally excluded given the HBA's inherent ethical foundation and the risk to violate legal requirements. (ii) Anticipated benefit is reframed as a secondary factor of project outcome. (iii) The total animal numbers are removed entirely, arguing that only the minimum number of animals necessary to achieve the intended outcome can be sensibly justified. The CM's feasibility in review settings remains to be determined. An empirical study of the CM's practical value will be outlined, exploring if persistent challenges in HBA practice can be meaningfully addressed through this approach.

Abstract Number: 16

Presentation

TOWARDS A TRANSPARENCY AGREEMENT FOR AUSTRIA – AN INTERIM REPORT

Auke Boersma[1], *Birgit Reininger-Gutmann*[2], *Roberto Plasenzotti*[2], *Andrea Heinzle*[2], *Victoria Schiffer*[2], *Maik Dahlhoff*[1]

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[2] The RepRefRed Society/Austrian 3R Center, Graz, Austria

Across Europe and beyond, Transparency Agreements (TAs) have proven to be effective instruments for fostering openness and constructive dialogue about the use of animals in biomedical research. Currently, eight European countries — Belgium, France, Germany, Portugal, the Netherlands, Spain, Switzerland, and the United Kingdom — have active national Transparency Agreements, complemented by comparable initiatives in Australia and New Zealand. Encouraged by the European Animal Research Association (EARA) and led by the RepRefRed Society, similar efforts are now emerging in Austria. Following discussions among a group of veterinarians who work with laboratory animals, the need arose to establish a TA in Austria that would be equivalent to those in other countries.

The proposed Austrian TA is based on four core commitments: creating clarity about animal use in research; improving communication with the media and the public; proactively informing and engaging in dialogue with society; and reporting annually on progress while sharing experiences. The overarching goal is to finalize an Austrian Transparency Agreement by the end of 2026 through a dedicated working group and to encourage as many public and private research institutions as possible to become signatories. This initiative aims to strengthen trust, promote informed public discourse, and align Austria with best practices in transparent animal research communication.

Abstract Number: 123

Presentation

OPENNESS IN ANIMAL RESEARCH: EUROPEAN EXPERIENCES WITH TRANSPARENCY AGREEMENTS

Kirk Jordan Leech[1]

[1] European Animal Research Association, London, United Kingdom

Open and transparent communication about the use of animals in biomedical research is increasingly recognised as a core component of responsible science. In response to evolving societal expectations and scrutiny, a growing number of European countries have adopted national Transparency Agreements (TAs) as voluntary frameworks through which research institutions commit to proactive communication about animal research.

Since the first TA launched in the United Kingdom in 2014, the adoption of TAs has expanded across Europe and beyond, bringing together universities, research institutes, funders and industry partners. While all agreements are built on shared core commitments, their implementation is adapted to national contexts, reflecting local practices and structures. This diversity has resulted in a range of approaches, from coordinated national campaigns to local initiatives such as open days, training programmes and narratives on animal use.

Across countries, these agreements have provided platforms for exchanging experiences and good practices, helping institutions address challenges such as public misconceptions and the complexity of explaining animal research. This has helped lower barriers to openness and increasing confidence in engaging with the public and policymakers, strengthening internal communication.

Taken together, Transparency Agreements illustrate how coordinated, voluntary approaches can support trust, dialogue and responsibility in animal research.

Abstract Number: 175

Presentation

AN OVERVIEW OF INTERNATIONAL AND EUROPEAN DEVELOPMENTS IN THE THREE RS AND NAMS FIELD

Winfried Neuhaus[1]

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The transition toward animal-free biomedical research and safety assessment is gaining momentum worldwide, driven by scientific innovation, evolving regulations, and policies promoting the 3Rs: Replacement, Reduction, and Refinement. This presentation highlights recent international and European developments in New Approach Methodologies (NAMs), focusing on policy initiatives and collaborative networks that support non-animal approaches.

Internationally, major policy and funding efforts are accelerating the integration of NAMs into biomedical research and regulatory science. In the United States, recent versions of the FDA Modernization Act and strategic initiatives by the National Institutes of Health (NIH) support the use of human-relevant in vitro and in silico methods in drug development and safety testing.

In Europe, the policy landscape is evolving rapidly. European Commission activities, including work on a roadmap to phase out animal testing for chemical safety assessments, reflect a strong strategic commitment to NAMs. Collaborative frameworks such as EPAA and EU3Rnet foster cooperation among regulators, industry, and academia. New EC and Horizon Europe initiatives, including the European Research Area on NAMs and the project NAMWISE, further promote regulatory uptake through guidance, training, and stakeholder engagement. Together, these efforts mark substantial progress in integrating NAMs into research and regulatory practice.

Abstract Number: 32

Poster

FROM WASTE TO RESOURCE: HIGH-QUALITY BIOBANKING OF SURPLUS MATERIAL FROM LABORATORY ANIMALS TO SUPPORT THE 3RS AT VETMEDUNI

Stefanie Burger[1], Regina Binder[2], Stefan Kummer[1], Melanie Stargardt[1], Monika Wieser[1], Ingrid Walter[1]

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Animal experiments routinely generate surplus biological material (e.g. tissues, biofluids) that is often discarded after completion of primary endpoints. Many researchers are unfamiliar with biobanking as a route for secondary use of these materials. To address this gap - and to support 3Rs, particularly Replacement and Reduction,- Vetmeduni's animal-use strategy advises project leaders to consult VetBiobank on biomaterial archiving options.

Sharing leftover materials from animals killed in experiments can increase research efficiency and reduce both the number of animals used in procedures and those killed solely to obtain biological material. Within BBMRI.at#2, the Austrian node of the European biobanking infrastructure (BBMRI-ERIC), VetBiobank established pilot cohorts of archived materials from animal experiments and evaluated their use.

The pilots demonstrated real-world feasibility and showed that high-quality sampling and controlled handling (standardized processing in accordance with pre-analytical ISO standards) yield samples "fit for purpose" for histology and molecular assays. Materials were used primarily for method establishment and had additional value for teaching. The archive particularly benefits investigators and educators who do not conduct animal experiments and otherwise lack access to biological material. Because cohort building is resource-intensive, demand-informed prioritization and sustained quality management are essential to maximize subsequent use.

Abstract Number: 142

Poster

THE WAY OF THE AUSTRIAN 3R CENTER

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The Austrian 3R Center (A3RC), established in 2020 by the RepRefRed Society and funded by the Federal Ministry of Education, Science and Research, serves as Austria's national knowledge platform for the 3Rs and related topics. Aiming to set up a strong national and international 3R community, A3RC organizes initiatives such as the Austrian 3R Days, online seminar series, and active engagement through social media. Beyond uniting the 3R community, the A3RC emphasizes the importance of connecting smaller national stakeholder groups, including animal welfare bodies in academia and industry, as well as laboratory veterinarians in Austria. The A3RC regularly organizes meetings for the animal welfare bodies and the laboratory veterinarians to facilitate knowledge exchange among these groups. Initially, the A3RC knowledge transfer mainly focused on scientific staff as its primary audience. However, recognizing the importance of inclusivity, the center is now expanding its efforts to engage a very important group of people, the animal caretakers across Austria, by setting up an exclusive online seminar series for animal caretakers. By establishing these networks, the A3RC is able to completely connect all the national game players in animal research with each other. This offers the opportunity to have a network all over the animal research landscape in Austria to distribute information in a more targeted way, which will drive forward innovation in the field of the 3Rs.

Abstract Number: 152

Poster

STEPPING IN FRONT OF THE CURTAIN - MAKING BIOMEDICAL RESEARCH TANGIBLE FOR PUPILS

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Transparency in animal testing will become more and more important in the future. But how should we handle this delicate topic? Which should be the first steps to become transparent? Where should we start? The division of Biomedical Research – the institution for animal research and alternative methods at the Medical University of Graz (Austria) started their own transparency project by actively inviting schoolchildren from an age of 14 - 17 years for workshops directly at the Medical University. Teachers are supplied with information material such as a Q&A folder, which was specially created for this purpose, in advance to prepare their pupils for the visit. During the workshop, the children are informed about animal testing, alternatives to animal testing and the 3Rs via presentations and are able to take part in a virtual tour through the animal facility. Afterwards they can test their pipetting skills, do some hand on trainings in sewing and blood withdrawal on dummy models and are able to talk to an animal caretaker to get information about a daily life of a lab animal. Children are our future researchers and we think it is important to start being transparent at an early age. The aim of this workshop is not to convince them what is right or wrong but to support them to set up their own point of view when dealing with a controversial topic like animal experiments. It is time for us to step out of the curtain!

Abstract Number: 157

Poster

ESTABLISHING A NATIONAL 3R CENTER IN TÜRKIYE: INTEGRATING INSTITUTIONAL EXPERTISE INTO A GLOBAL 3R FRAMEWORK

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The principles of Replacement, Reduction, and Refinement (3Rs) are central to responsible, high-quality biomedical research. As global standards increasingly emphasize animal-free and human-relevant methodologies, Türkiye has a timely opportunity to establish a coordinated national 3R framework.

At Istanbul Medipol University's SABITA, we combine expertise in rodent and axolotl models with alternative research platforms, including Drosophila, transgenic cell lines for drug screening, and chick chorioallantoic membrane (CAM) assays. In parallel, we develop human-relevant in vitro and in silico systems such as organoids, organ-on-chip models, and omics-based approaches. This integrated portfolio enables us to connect in vivo expertise with innovative animal-free methodologies. Through the Türkiye 3R Campus initiative, we aim to provide shared infrastructure, structured training, and evidence-based evaluation of experimental strategies across institutions.

A National 3R Center would reduce fragmentation in current practices, harmonize standards with European frameworks, and enhance translational research quality. It would not only support refinement and reduction but actively promote replacement through scalable cell-based systems and alternative organisms.

By strengthening national coordination and fostering international collaboration, this initiative aims to position Türkiye within the global movement toward innovation-driven and ethically responsible biomedical research.

AI & ITS APPLICATION IN RESEARCH

Abstract Number: 178

Presentation

RECONSTRUCTING BRAIN TISSUE AT SYNAPTIC RESOLUTION WITH LIGHT MICROSCOPY

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The brain's ability for information processing and body control hinges on the highly complex arrangement of neurons into a functional network, with each neuron connected to others via thousands of synapses. Deciphering the cellular architecture of brain tissue at the level of synaptic connections, i.e. deriving connectomes at synaptic detail, requires nanometer scale resolution, comprehensive structural contrast, and the ability to image across spatial scales. Volume electron microscopy has been the technological basis for reconstructing connectomes, but obtaining molecular information is challenging and the required instrumentation is only available to dedicated labs. Light microscopy provides major opportunities for integrating molecular and functional information into circuit reconstructions, but has been limited to reconstructing sparse subsets of cells.

I will discuss our development of light microscopy based connectomics (LICONN) [Nature, 2025]. LICONN is based on specifically engineered hydrogel expansion providing the resolution and volumetric imaging required for tracing even thin neuronal structures and for reconstructing tissue at synaptic resolution using deep-learning methods. LICONN is compatible with multicolour imaging of specific molecules while imaging proceeds with standard off-the-shelf microscopes, facilitating adoption of the technology. I will highlight our efforts towards increasing molecular information content for multimodal mapping of brain tissue.

Abstract Number: 172

Presentation

IN SILICO HAZARD ASSESSMENT - FROM QSAR TO DEEP LEARNING AND AI

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Computational models such as QSAR are widely used to predict biological activity and toxicological endpoints and thus contribute to the replacement and reduction of animal experiments. Traditional QSAR models rely mainly on 2D chemical structures and are typically developed for single targets. With the increasing availability of 3D-protein strucutres, structure-based methods such as molecular docking and molecular dynamics simulations allowed mechanistic insights into compound-target interactions in 3D space. In parallel, large public data sources such as ToxCAST and ChEMBL enable deep learning approaches, such as DeepTox and our multitask deep neural network for simultaneous prediction of 78 off-targets. However, many current approaches still neglect the broader biological context. Therefore, we developed models for prediction of hepatotoxicity incorporating compound-target and compound-pathway fingerprints. This both improved predictive performance and allowed the identification of novel potential molecular initiating events, thereby supporting mechanism-based toxicity prediction within the 3R framework.

SPOTLIGHT ON REDUCTION IN PRECLINICAL SETTINGS

Abstract Number: 41

Poster

SEEING MORE WITH LESS: PRECLINICAL IMAGING AS CONTRIBUTOR TO REDUCTION AND REFINEMENT STRATEGIES IN ANIMAL STUDIES

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Preclinical imaging plays a central role in the reduction and refinement principle of the 3Rs concept. In vivo imaging techniques allow us to observe natural and induced processes and diseases in living animals non-invasively and over extended periods.

Preclinical imaging methods offer high accuracy and reproducibility, leading to an improved understanding of functional and molecular processes by monitoring dynamic and sensitive biological processes over time. This results in the gain of important information while simultaneously reducing the number of animals.

Using state-of-the-art techniques, our Core Facility can generate images at various levels, from whole-body images to subcellular structures. The following methods are currently available for this purpose: Non-invasive to minimally invasive imaging using micro-CT, ultrasound, and optical imaging based on fluorescent or bioluminescent techniques, provide answers to specific questions, both individually and as a complementary approach.

In summary, the generation of high quality, reproducible data can thus be reconciled with a reduction in the number of animals used, ultimately contributing to the high ethical standards for the treatment of laboratory animals.

Abstract Number: 45

Poster

CANINE ORGANOIDS-ON-CHIP FOR DRUG DISCOVERY – THE PARACETAMOL CASE STUDY

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The pharmaceutical industry is shifting toward animal-free preclinical testing, guided by the 3Rs principles. Organoids integrated with microfluidic technologies stand out as scalable, physiologically relevant models that better connect 2D cell cultures to in vivo conditions.

This work spotlights canine liver organoids - an underutilized yet potent tool for investigating drug metabolism and toxicity in dogs as patients. Dogs feature prominently in pharmaceutical research due to metabolic parallels with humans, but their species-specific sensitivities, like heightened acetaminophen (paracetamol) vulnerability, lack mechanistic clarity. We seek to uncover these differences.

Our platform features patient-derived canine liver organoids from veterinary cases, cultured in a custom 32-chamber microfluidic chip designed for standard multiwell plate compatibility. It enables continuous perfusion, maintains tissue architecture, and produces passive gradients ideal for drug response studies.

We assess dose- and time-dependent paracetamol exposure under static vs. dynamic flow, measuring viability and glutathione levels alongside live-cell imaging of apoptosis and necrosis. Complementary LC-MS proteomics and qPCR analyses profile metabolic enzymes and oxidative stress pathways, elucidating biotransformation in canine patient-derived cells.

This organoid-on-chip advances translational safety assessment for canine patients, minimizing animal use while revealing species-specific mechanisms.

Abstract Number: 64

Poster

ESTABLISHMENT OF ORGANOTYPIC SLICE CULTURES FROM HUMAN, PORCINE AND MURINE CORTICAL TISSUE AS A TRANSLATIONAL PLATFORM TO MODEL HUMAN BRAIN DISEASE

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Organotypic brain slice cultures (OTC) provide an in vitro platform to study human neuropathology while reducing in vivo animal use. We aim to establish and optimize OTC from human, porcine and murine cortex to compare their suitability as translational models. Human tissue is obtained from epilepsy and peritumoral surgeries, porcine brains (rostrum gyrus) from the slaughterhouse. For murine tissue, sensorimotor cortical slices from one brain can be used for several experiments, maximizing experimental output per animal and thereby reducing the number of animals required. OTCs are maintained on semi-permeable membranes for up to 14 days. Structural preservation and cellular composition are evaluated by immunofluorescent labelling for neurons (NeuN), neuronal compartments (voltage-gated ion channels, cytoskeletal and other structural proteins), microglia (Iba1) and astrocytes (GFAP). After 7 days in culture, human and porcine OTCs show preserved cortical layers and Iba1-positive microglia. To comprehensively assess tissue health across all three species, we will implement LDH assays and qPCR analyses of genes linked to neuronal integrity, glial activation, and stress responses. This framework supports the feasibility of generating stable human, porcine and murine OTC, highlights porcine by-product tissue, and efficiently used murine tissue as ethically and structurally valuable complements to human models.

Abstract Number: 136

Poster

A 3D PLATFORM FOR THE ASSESSMENT OF CHONDROGENESIS IN VITRO: ADVANCING THE 3RS IN ENGINEERED CARTILAGE

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Osteoarthritis affects millions and drives substantial clinical and economic burdens. Preclinical evaluation of engineered cartilage is often hampered by non-standardized workflows and limited in vitro readouts. We aim to develop a microperfused 3D new approach methodology (NAM) that reliably matures engineered cartilage and provides real-time, non-destructive monitoring of chondrogenic differentiation, thereby improving translational relevance and refining, reducing, and replacing animal testing. Umbilical cord derived human mesenchymal stem cells (uc-hMSCs) will be encapsulated in cartilage-mimetic hydrogel scaffolds and interfaced with an open-flow microperfusion (OFM) catheter incorporating biosensors for continuous metabolite monitoring. A validated dual-circuit perfusion bioreactor-comprising a bulk medium loop and a microperfusion loop operating at <1 µL/min that equilibrates with interstitial fluid via the OFM probe that will enable real-time, sequential metabolite analysis to resolve intraconstruct gradients not captured by supernatant assays. Standardized culture regimes will be compared to optimize chondrogenesis, integrating online metabolic profiling with histological and biochemical endpoints. By establishing data-driven thresholds for differentiation and functional performance, the system will provide a robust in vitro decision framework to evaluate constructs before any animal use and thereby decreasing the number of animals needed for confirmatory experiments

SPOTLIGHT ON REFINEMENT: 3RS IN ANIMAL FACILITIES

Abstract Number: 15

Poster

REPLACEMENT OF SENTINEL MICE BY ENVIRONMENTAL HEALTH MONITORING (EHM) IN LABORATORY MOUSE FACILITIES AND FACTORS INFLUENCING ITS IMPLEMENTATION IN GERMAN-SPEAKING COUNTRIES

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The health status of laboratory animals plays a decisive role for their health and welfare as well as the validity of the study results. In recent years, numerous comparative studies demonstrated that environmental health monitoring (EHM), which detects nucleic acids of infectious agents via molecular biological methods, is superior to soiled bedding sentinels (SBS). Its implementation can reduce the number of mice used for health monitoring (HM) in conformity with the 3Rs. A survey containing 33 questions was conducted by members of the Committee for Hygiene of the GV-SOLAS to assess the prevalence of EHM use in Germany, Austria and Switzerland and to better understand factors that influence its implementation in laboratory animal facilities.

Our survey showed that 64% of 91 animal facilities predominantly use SBS for their HM programs, 20% use a combination of EHM and animals, and 16% implement EHM. Notably, 59% of facilities equipped with at least 50% IVCs already use EHM to varying degrees. An annual reduction of 8-1,200 animals per facility was reported when EHM was used. Beliefs on factors such as cost, reliability of the methodology, and the number of false-positive results differ among facilities. The choice of HM strategy is influenced by the existing cage system and the availability

of decontamination equipment. Furthermore, inclusion of EHM in the FELASA recommendations, more information, and further training on EHM will most likely enhance the acceptance of EHM.

Abstract Number: 18

Poster

VALIDATION OF A SEDATION SCALE FOR MICE AND DEVELOPING A PROTOCOL FOR ABDOMINAL MRI AND THERMOGENESIS EVALUATION

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During diagnostic imaging, anesthesia is required to immobilize the animal and ensure data acquisition. In mice, the small size and high metabolism can be a challenge due to motion artifacts. The aim of Phase 1 is to evaluate the protocols and validate the sedation scale already stablished for rats, for mice. Developing a sedation scale would facilitate the evaluation and help providing guidelines. The objective of Phase 2 is to evaluate the protocols for metabolic research. As a readout, we examine brown adipose tissue thermogenesis. Protocol 1:Pentobarbital 60 mg/kg. Protocol 2:Dexmedetomidine 25 µg/kg/ midazolam 2mg/kg /Butorphanol 5 mg/kg. Protocol 3:Medetomidine 50 µg/kg/ midazolam 2mg/kg/ Butorphanol 5 mg/kg, all IP. Phase 1, we will compare protocols 2 and 3, and validate the sedation scale for mice. In Phase 2.1, MRI will be performed, this will enable evaluation of interscapular brown adipose tissue temperature, lipid consumption, and blood perfusion. In this phase there will be a comparison of the sedation protocols with protocol 1 for MRI acquisition. In phase 2.2 we will perform the assessment of sedation effects on the autonomic nervous system via β3-adrenergic-induced non-shivering thermogenesis using injection of CL 316,243 (1mg/kg). This study can stablish better sedation, it could also refine future protocols according to the experiment. Moreover, using better sedatives for MRI procedures will make the image acquisition faster and more accurate.

Abstract Number: 21

Poster

USE OF THE SEDATIVUM TRAZODONE TO REDUCE STRESS DURING ANESTHESIA IN MICE

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The sedativum Trazodone (Trittico, Aziende Chimiche Riunite Angelini Francesco, Italy) is applied in humans and pet animals to reduce the burden prior to and during anesthesia or just in order to reduce any kind of stress. In mice it is also supposable as refinement (3Rs) in surgical procedures. Therefore, we applied together with analgesia either a low dose Trazodone (25mg/kg), a high dose (50mg/kg) or a vehicle (NaCl, H2O), anesthesized pseudopregnant mice with two different methods (Sevofluran, Ketamin/Xylazine) and conducted sham embryo transfers. During anesthesia heart and breathing rate was recorded with a MARTA device for non-invasive monitoring of vital parameters. Before and after the procedure fecal pellets were collected in order to determine fecal glucocorticoid metabolites as indicator for experimental stress.

Application of both doses of Trazodone did not reduce the amount of stress metabolites measured in the feces. Nevertheless, breathing and heart rate were affected. The anesthetic dosage of Sevofluran could be reduced after induction in order to keep anesthesia deep enough for surgical tolerance and breathing and heart rate at a constant level. With the injectable anesthesia breathing and heart rate went further down and anesthesia with the standard dose according to body weight was prolonged. Nevertheless, trazodone administration may reduce spontaneous excitations in mice prior to anesthesia, which occur at a rate of 0.1–5% depending on the laboratory.

Abstract Number: 37

Poster

ANIMAL FACILITY UNDER CONSTRUCTION: EFFECTS ON THE CONCENTRATION OF FAECAL CORTICOSTERONE METABOLITES OF LABORATORY RODENTS

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Stress related health problems are a major restriction in the welfare of lab animals. There are numerous studies regarding stress signs and welfare problems caused by high noise levels and ambient vibrations. The aim of our study was the analysis of the stress level of mice in a laboratory animal facility during construction work compared to quiet surroundings. The level of feacal corticosterone metabolites (FCM) of 120 cages with 2 female mice per cage of 12 different mouse strains and additional 50 cages with 1 male mouse was analysed. The results showed significantly different FCM levels during construction times compared to quiet surroundings in most mice strains. Our work shows the possible influence of loud noise and vibrations on the stress level of laboratory rodents and could perhaps be used as a reference point for stress reduction during construction work in the future.

Abstract Number: 81

Poster

EQUIVALENCE-BASED ASSESSMENT OF ROBUSTNESS OF HOME-CAGE BEHAVIOURS IN MICE

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Home-cage behaviours, such as burrowing or nesting, are increasingly used to assess pain-associated changes in mice. Behavioural testing outside of the home cage is known to require a high degree of standardization in test setups to generate consistent data. This has not yet been systematically investigated for home-cage behaviours, although it might be of significant relevance for calculating the number of animals required and the study design. We therefore retrospectively examined a comprehensive data set collected from six different untreated mouse cohorts (21 male and 21 female C57BL/6J mice, each) under standardized conditions in the same laboratory at two trials three weeks apart. Equivalence testing (using 90% confidence intervals), based on WHO guidelines for bioequivalence in drug development, was adapted to assess robustness. Thus, food consumption was robust in both sexes, whereas water intake was robust only in males. In contrast, wheel running activity was robust only in females. Burrowing and nesting data did not meet robustness criteria in either sex. In consequence, only with robust parameters subtle changes can be determined with reasonable numbers of animals.

Abstract Number: 99

Poster

CRYOPRESERVATION – AN INDISPENSABLE TOOL FOR REDUCTION AND REFINEMENT IN MOUSE FACILITIES

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The cryopreservation of genetically modified mouse lines offers substantial opportunities to advance both Reduction and Refinement, by significantly reducing animal numbers, avoiding the production of surplus animals and the transport of live animals. This is particularly important for lines associated with phenotypic burden that should be maintained only when actively required for experiments. The Unit of Laboratory Animal Medicine at Vetmeduni is involved in developing cryopreservation techniques as part of the European Mouse Mutant Archive (EMMA; www.infrafrontier.eu/emma), which offers cryopreservation free of charge for scientists worldwide.

Beyond long-term storage, cryopreservation enables mouse lines to be shipped as frozen sperm or embryos, thereby eliminating transport-related stress. Less widely known is the fact that both embryos and mouse epididymides can be shipped refrigerated at 4 °C for several days and successfully

transferred or cryopreserved at the receiving institution. Furthermore, in vitro fertilization and blastocyst genotyping allow genetic quality control without generating live animals. Finally, robust and simple cryopreservation techniques are available for emergency situations—such as the COVID-19 pandemic—allowing the rapid safeguarding of numerous mouse lines without specialized equipment or liquid nitrogen. Together, these approaches highlight cryopreservation as a powerful yet underutilized tool to advance the 3Rs in mouse research.

Abstract Number: 127

Poster

CHARACTERIZING SUBCUTANEOUS METAMIZOLE IN RATS TO INFORM REFINEMENT OF PAIN MANAGEMENT STRATEGIES: PHARMACOKINETICS, SAFETY, AND ANTI-NOCICEPTIVE EFFICACY

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Metamizole (MET) is widely used in laboratory rodents to treat mild to moderate pain. However, data on pharmacokinetics, safety, and antinociceptive efficacy is scarce. MET (100-300 mg/kg, s.c.) was injected to healthy adult male and female RjHan:SD rats (n=9-21). Blood was sampled, and metabolite levels (4-MAA, 4-methylaminoantipyrine; 4-AA, 4-aminoantipyrine) determined. Safety was evaluated by clinical scoring, Irwin test, and recording of bodyweight and body temperature. The tail immersion test served to examine antinociceptive efficacy.

Plasma levels peaked at 0.5 h for 4-MAA and at 3 h for 4-AA. Body temperature was decreased in females for up to 2 h (250 mg/kg, p<.0001) and in males at 2 h (100, 200, 300 mg/kg: p=.0015; p=.0050; p=.0001). The Irwin test showed transient sedation and autonomic reactions and rat grimace scale action units were impacted. Plasma metabolite levels correlated with side effects, e.g. 4-MAA concentration with sedation sum score in males (p=.0193, r=.4556). The tail immersion test in males at 2 h post MET showed an increased latency to tail withdrawal vs. baseline (300 mg/kg, p=.0204), and the latency correlated with 4-AA (p=.0100, r=.4239).

The species-specific half-life of MET metabolites is comparatively short, and antinociceptive effects were mainly noticed at doses higher than currently recommended. Due to the observed side effects, MET cannot be recommended for repeated treatment in rats, but it may still be useful for managing acute pain.

Abstract Number: 135

Poster

LIMITATIONS OF STANDARD OPIOID ANALGESIC TREATMENT IN C57BL/6J MICE: IMPLICATIONS FOR ANALGESIA REFINEMENT

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Evidence-based improvement of analgesia contributes to the 3R refinement principle in laboratory animal studies. Opioids such as buprenorphine (BUP), butorphanol (BUT), and tramadol (TRA) are frequently used, but their pharmacological properties in mice remain insufficiently characterized. We therefore compared pharmacokinetics, anti-nociceptive effects, tolerability and potential impacts on home-cage behavior in healthy C57BL/6J mice (21 per sex and drug). After s.c. injection at recommended doses, maximum plasma levels (LC-MS/MS) were reached rapidly, with short elimination half-lifes of around 1-2 h. BUP and BUT produced transient anti-nociceptive effects in the tail immersion test 1 h after injection. All opioids caused temporary hyperthermia and signs of excitation and impaired coordination in the Irwin test. Administration via drinking water for five days achieved continuous target dose intake, but plasma levels remained below estimated therapeutic thresholds, and no anti-nociceptive effects were detected. Wheel running activity decreased, whereas food/water intake, body weight, burrowing, nesting, blood parameters and organ histopathology remained largely normal. TRA induced increases in liver enzymes. These findings suggest that current opioid dosages may not provide continuous analgesic coverage in mice. For s.c. administration, extended-release formulations appear advisable, while oral treatment may benefit from combination with other analgesics, such as carprofen.

Abstract Number: 140

Poster

3R IN ACTION-EINE DIREKTE UMSETZUNG DER 3R-PRINZIPIEN AN DER BIOMEDIZINISCHEN FORSCHUNG IN GRAZ

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The significant and ongoing problem of surplus animals in animal experimentation has been well-documented. Consequently, the Biomedical Research Department at the Medical University of Graz has devised an internal programme that directly implements the 3R principles to minimise the number of animals used. The utilisation of the fictional animal owner "3R in Action" in the animal management program PyRAT facilitates the "release" of unneeded, unburdened animals as 3R animals. These animals are recognised in the system as animals to be rehomed and can then be passed on to other research groups. This approach serves to minimise the number of unused surplus animals and sacrifices. Moreover, this method of animal transfer has the additional advantage of reducing costs and the time required for the acquisition of new animals.

Abstract Number: 141

Poster

RAT PLAYPEN: A REFINEMENT EASY TO IMPLEMENT

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It is well established that rats frequently demonstrate an inability to express their full behavioural repertoire in standard cages. It is evident that a multitude of behaviours cannot be fully expressed due to the limited space available. Consequently, the Biomedical Research at the Medical University of Graz has established a method of rat playpens that has achieved widespread acclaim and has been adopted for routine use. At regular intervals and in consultation with the researcher responsible, the rats are permitted to spend a few hours in a specially designed rat playpen. In this environment, the animals are encouraged to engage in natural behaviours and interact with other members of their group, thereby facilitating social interaction. It is evident that significant progress has been made in the management and containment of the animals. The utilisation of playpens as a training or socialisation aid for animals exhibiting incompatibility has also been seen. This method constitutes a straightforward approach to implementing an enrichment procedure that is both visually effective and "playfully" straightforward to execute.

Abstract Number: 143

Poster

SYRINGE FEEDING—AN ANIMAL-FRIENDLY ALTERNATIVE TO ORAL GAVAGE

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It is well-documented that oral gavage in laboratory animals is known to cause stress reactions and discomfort, especially when the procedure is repeated. In order to reduce stress and improve the well-being of animals, a new approach to oral administration was tested at the Biomedical Research of the Medical University of Graz as an alternative method to oral gavage in rats. The substance to be administered is mixed into condensed milk and offered directly to the rat to drink using a syringe with a special attachment. Following a brief period of hesitation, the animals readily accepted and voluntarily consumed both pure condensed milk and condensed milk mixed with medication. This method is a promising approach to reducing or avoiding oral gavage in laboratory animal experimentation.

Abstract Number: 146

Poster

HOME-CAGE STEREOTYPIES BIAS PERFORMANCE IN STANDARD MOUSE BEHAVIORAL TESTS FOR TRANSLATIONAL PSYCHIATRY RESEARCH

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The reproducibility of mouse models in translational psychiatry is frequently undermined by environmental factors, yet the extent to which husbandry-induced stress biases experimental outcomes, remains poorly quantified. Standard laboratory housing often exceeds the adaptive capacity of mice, leading to the development of abnormal repetitive behaviors (ARBs). However, conventional detection of these stereotypies relies on brief, labor-intensive manual scoring of video samples, which lacks the temporal resolution to capture subtle behavioral shifts or their longitudinal impact on data variability. Here, we present a continuous, non-invasive, observer-free approach that detects circling behavior using a deep-learning algorithm applied to home-cage activity data. This method reliably detected circling, with females, particularly when singly housed or kept under minimal enrichment, showing the highest circling levels. Circling predicted performance in standard tests probing social interaction, anxiety-like behavior, and behavioral despair, demonstrating that even low levels of stereotypy introduce bias of phenotypic readouts. Animals with elevated circling showed brain molecular signatures consistent with chronic stress and increased anxiety. Overall, these findings indicate that home-cage stereotypies are not merely indicators of compromised welfare, but can systematically alter behavioral and molecular outcomes in translational psychiatry research.

Abstract Number: 147

Poster

GUIDED TOURS IN A LABORATORY ANIMAL HUSBANDRY POSITIVELY CHANGE THE ATTITUDE TOWARDS ANIMAL TESTING

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On 24.4.2024, World Day for Laboratory Animals, the Center for Biomedical Research opened its doors to give MedUni Vienna employees an insight into a laboratory animal husbandry. The tour aimed to offer transparent information on animal testing, especially focusing on husbandry conditions. Accompanied by the tour, a survey was conducted, asking the participants about their general opinion on animal testing, their expectations of the husbandry conditions as well as their opinion of the personnel working with laboratory animals before as well as after the tour. In general, it was asked about their opinion on transparent information about animal testing.

3 tours of 15 people each were conducted and 30 people completed the survey. The tours were led by the veterinarians responsible and consisted of a conventional mouse room, the rat husbandry, finishing at the pigs unit. Highlights were the alternative rat housing system of the Medical University of Vienna as well as the pigs that showed their curious, open and playful character, actively approaching the visitors.

The results of the survey were very positive, all parameters were assessed significantly more positive after the tour than before. Especially the opinion on the personnel working with laboratory animals changed drastically after the tour. This clearly shows that lay people are not only very interested but also open about animal testing if approached in an open and transparent fashion.

Abstract Number: 150

Poster

G3RP: GOOD 3R PRACTICE - QUALITY MANAGEMENT AS A BASIS FOR ANIMAL WELFARE

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Quality Management Systems provide a robust structural framework to foster a Culture of Care in animal experimentation. But where to start? The complexity and fear of implementing a QMS often acts as significant barrier –and the hurdles seem far too difficult to overcome setting up an adequate system. To address this, the Austrian 3R Center has developed a consulting tool designed to bring more quality and animal welfare into animal facilities.

Our approach is based on standard quality management methodologies and offers a tailored consultation. The G3RP Consulting Process begins by defining clear goals and conducting a thorough gap analysis to assess the current state. Whether focusing on a single experiment or an entire animal facility, this tool pinpoints areas for enhancement and offers practical, actionable guidance? With this tool, we can support taking the first steps towards implementing foundational elements of a QMS, as well as closing the gap between ISO, GxP, and other standards.

Our ongoing support ensures continuous improvement and alignment with the 3Rs principles.

The Austrian 3R Center´s Gap Analysis (G3RP) is based on the principles of the 3Rs (Replacement, Reduction, Refinement) but takes a more holistic approach to ensure reproducibility and data robustness.

Tested in 3 Austrian animal experimental facilities the G3RP Gap Analysis provides an easy tool for the implementation of quality standards in Lab animal research labs and animal facilities.

Abstract Number: 156

Poster

RAT JACKETS – A REFINEMENT IN ANIMAL EXPERIMENTATION

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In the realm of animal research, there exists a multitude of applications that necessitate the immobilisation and restraint of animals over extended periods. One such example is experiments involving hormone pumps (e.g., insulin pumps) or sensors attached to the body, which often pose a problem in rodents due to their high level of movement. In response to a request from a research group to test a hormone pump on freely moving rats, an animal keeper at the Biomedical Research Department of the Medical University of Graz was able to design and sew rat jackets from neoprene by hand. In preliminary trials conducted on practice animals, the jackets were found to be effective, with the animals acclimatising to them with ease. The jackets represent a prime example of applied refinement in the field of animal testing, with the potential to minimise both stress and animal discomfort. These devices facilitate the normal behaviour and movement of the animals during hormonal pump testing.

List of Speakers

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